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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/035,977	12/26/2001	Luc Desnoyers	P3030R1C10	4528

7590 09/15/2005

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OIP/IA/P

SEP 30 2005

EXAMINER

KOLKER, DANIEL E

ART UNIT PAPER NUMBER

1649

DATE MAILED: 09/15/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.		Applicant(s)	
	10/035,977		DESNOYERS ET AL.	
	Examiner		Art Unit	
	Daniel Kolker		1649	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) ☒ Responsive to communication(s) filed on 6/13/05, 7/1/05.
- 2a) ☐ This action is **FINAL**. 2b) ☒ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

- 4) ☒ Claim(s) 22 - 30, 32 - 35, and 38 - 41 is/are pending in the application.
- 4a) Of the above claim(s) _____ is/are withdrawn from consideration.
- 5) ☐ Claim(s) _____ is/are allowed.
- 6) ☒ Claim(s) 22 - 30, 32 - 35, and 38 - 41 is/are rejected.
- 7) ☐ Claim(s) _____ is/are objected to.
- 8) ☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☐ The drawing(s) filed on _____ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) ☒ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

- 12) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☐ All b) ☐ Some * c) ☐ None of:
1. ☐ Certified copies of the priority documents have been received.
2. ☐ Certified copies of the priority documents have been received in Application No. _____.
3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

- | | |
|--|---|
| 1) <input checked="" type="checkbox"/> Notice of References Cited (PTO-892) | 4) <input type="checkbox"/> Interview Summary (PTO-413)
Paper No(s)/Mail Date. _____ |
| 2) <input type="checkbox"/> Notice of Draftsperson's Patent Drawing Review (PTO-948) | 5) <input type="checkbox"/> Notice of Informal Patent Application (PTO-152) |
| 3) <input checked="" type="checkbox"/> Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08)
Paper No(s)/Mail Date <u>6/13/05</u> . | 6) <input type="checkbox"/> Other: _____ |

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DETAILED ACTION

1. Applicant's remarks, amendments, and declarations filed 13 June and 1 July 2005 have been entered. Claims 31, 36, and 37 have been cancelled; claims 22 – 30, 32 – 35, and 38 – 41 are pending and under examination.
2. The Art Unit location of your application in the USPTO has changed. To aid in correlating any papers for this application, all further correspondence regarding this application should be directed to Art Unit 1649.
3. The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.
4. The deletion of inventors under 37 CFR 1.48(b) is accepted.

Information Disclosure Statement

5. The information disclosure statement filed 13 June 2005 has been considered. The BLAST results indicate that applicants are aware of nucleic acids and proteins with relevant to examination. However the results cannot be considered because there is no alignment provided, nor is there an indication of the percent identity between the claimed sequence and the reference sequences. Applicant states on p. 9 of the remarks that the newly-submitted documents include references to specific accession numbers and sequences. Applicant is advised that the BLAST results submitted appear to be the results of a search and include a list of sequences which match, but do not provide either alignments or indications of how the sequences are related to the instantly-claimed peptides. Therefore the examiner cannot determine if the sequence accession numbers submitted by applicant constitute prior art. Furthermore the search results submitted appear to be the results of a search and are not publicly available documents. Applicant is directed to MPEP 609 and 37 CFR and 1.98 (a) – (d).

Withdrawn Objections and Rejections

6. The following objections or rejections made in the previous office action are withdrawn:
The objections to the specification. Applicant has deleted the hyperlinks and changed the title.

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The rejection of claims 22 – 27 and 34 – 35 under 35 USC 112, first paragraph, for lacking enablement as far as it relates to deposit of biological material. Applicant's declaration filed 13 June 2005 is sufficient to overcome the rejection.

The rejections under 35 USC 112, second paragraph. Applicant's amendments are sufficient to overcome the rejections.

Rejections Maintained

Priority

7. In the previous office action the examiner determined that the effective filing date for this application is the date the international application was filed, 1 March 2000. Applicant did not traverse this determination and thus it stands for the reasons made of record.

Claim Rejections - 35 USC §§ 101 and 112

8. Claims 22 – 30, 32 – 35, and 38 – 41 are rejected under 35 U.S.C. 101 because the claimed invention is not supported by either a specific and substantial asserted utility or a well established utility.

The rejection is maintained for the reasons made of record in the previous office action and reiterated below.

The claims are drawn to isolated nucleic acids encoding polypeptides, called PRO4380, as well as variants at least 80% identical thereto fragments of same, as well as nucleic acids which comprise same and vectors and host cells. The specification asserts that PRO4380 has two specific utilities, as it came up positive in two assays, however neither utility is substantial.

Applicant did not address the examiner's arguments that the first assay, (Example 37, page 166), which discloses compounds that test positive as either stimulators or inhibitors of glucose or FFA uptake, does not constitute a patentable utility. This assay is deemed to lack utility for the reasons made of record in the previous office action, p. 4 second complete paragraph – p. 5, first complete paragraph).

The data presented in Example 41 (p. 168 – 169) of the specification indicate that PRO4380 was positive in the Mouse Kidney Mesangial Cell Proliferation Assay. It is acknowledged that proliferation of mammalian kidney mesangial cells is useful as Schocklmann (1999. Kidney International 56:1199-1207) teaches that such proliferation is necessary after injury or damage to the kidney. However, the threshold used in determining whether a

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particular PRO molecule counts as “positive” in this assay would not be considered reasonable by one of skill in the art. The specification discloses (p. 169, lines 1 – 2) that positives in this assay include anything which is at least 15% over the control reading. The post-filing publication by Rovin et al. (2002. *Kidney International* 61:1293-1302) indicates that a 21% increase in human mesangial cell proliferation is not statistically significant (see particularly p. 1296, lines 3 – 6).

On page 10 of the remarks applicant refers to the utility guidelines on specific, substantial, and credible utilities. The claims were not rejected for lack of credible utility, thus arguments related to this point (i.e page 10, point (3) in the final paragraph) are not germane. Particularly, the citation of MPEP 2107 II (B)(1)(ii), drawn to credible utilities is not on point as no rejection for lack of credibility was made.

On p. 11 of the remarks, applicant argues that utility need not be proven, that a reasonable correlation between the evidence and the asserted utility is sufficient, and that the asserted utility should be accepted if it is more likely than not true. Applicant cites *In re Langer*, *In re Jolles*, *In re Irons*, *In re Sichert*, *Raytheon v. Roper*, and *In re Oetiker* as supporting this argument. Applicant’s arguments have been fully considered but are not persuasive.

In *In re Langer*, the court ruled the Patent Office cannot require clinical testing in humans to rebut a prima facie case for lack of utility. In the instant case, the Office has not made such a requirement. Furthermore the Langer court ruled that “Assuming that sufficient reason to question the statement of utility and its scope does exist, a rejection for lack of utility under § 101 will be proper on that basis; such a rejection can be overcome by suitable proofs indicating that the statement of utility and its scope as found in the specification are true.” In the instant case there is in fact sufficient reason to question the statement of utility. The reference by Rovin cited in the previous office action indicates that the 15% threshold used by applicant is not reasonable. Therefore one of skill in the art would have reason to doubt the asserted utility.

In *In re Jolles*, the issue was whether data from an art-recognized animal model could be considered predictive of results in humans. That is not an issue in the instant case, as the examiner indicated in the paragraph spanning pp. 5 – 6 of the previous office action that proliferation of mammalian kidney mesangial cells would be useful, as it is an important part of the repair process after injury (see Schocklmann, particularly pp. 1199 - 1200 for more detailed discussion). But since the threshold used by applicant was 71% of a change shown not to be

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statistically significant, one of skill in the art would conclude that there PRO4380 does not induce any more mesangial cells than are induced under control conditions.

The citation of *In re Irons* is also not relevant to the instant case. In *Irons*, evidence was submitted that indicated that the drug had been administered to 888 patients and that statistically significant results were obtained showing an improvement in arthritic conditions. In the instant case, no such evidence has been submitted. In the instant case, the claimed product has not been administered to patients. Furthermore, there is no evidence of record indicating a statistically significant result *in vitro*.

The *Sichert* court ruled that blind comparative studies of the claimed compositions, which showed that the compositions were effective in relieving lymphatic congestion (as narrowly defined), were sufficient to establish utility of said compositions under 35 USC § 101. In the instant case, applicant has not shown any such studies, and therefore because the fact pattern is sufficiently different the *Sichert* case is not germane.

In *Raytheon v. Roper*, utility was found by the Federal Circuit when a lack of utility had been found by a lower court. This was due not to the sufficiency of the evidence presented, but rather because the Federal Circuit ruled that the claims in question had been interpreted erroneously. In the instant case, there does not appear to be a question as to how the pending claims are being interpreted.

It is not immediately apparent why applicant has cited *In re Oetiker* in arguments related to the utility under 35 USC § 101, as the *Oetiker* case dealt not with utility but with obviousness under 35 USC § 103. No claims have been rejected under § 103 in the instant case.

The examiner acknowledges that the ability to induce mesangial cell proliferation is specific. However, the assay used by applicant and reported in Example 41 beginning on p. 168 of the specification would not allow a skilled artisan to conclude that it is more likely than not that the asserted utility is true and therefore the asserted utility is not substantial.

On p. 12 of the remarks applicant discusses the examiner's interpretation of the results from Rovin. The examiner and applicant appear to agree on this point. Rovin clearly stated that the data point, a 21% increase, did not represent a significant difference due to the large degree of variability inherent in this assay. Because of the large degree of error, a 21% increase is not significant. Stated another way, one of skill in the art would recognize that it is *improper* to conclude that the two samples (control and 5 uM ciglitazone) are drawn from different populations.

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Significance does not mean, as applicant asserts on p. 12, final paragraph, of the remarks, that there is not an overlap of standard deviations or errors in the data set. Rather, statistical significance is a determination, based on mathematic procedures, that the observed difference between samples has a less than 5% chance of occurring by random accident (see attached definition from the On-line Medical Dictionary, accessed 22 July 2005). Applicant argues that Rovin's report of a 21% non-significant difference indicates that the statistical error in their measurement overlaps with the statistical error in the control set, and that "this does not mean that an increase of proliferation of 21% is not scientifically important or significant, but means that Rovin's particular measurement of 21% may be incorrect due to the amount of error for that data point." This is not the way significance is understood in the art. Significance is an inference. When a result reaches statistical significance, it is proper to infer that the two samples are drawn from separate populations. When the result is not significant, the appropriate inference is not that the particular value is subject to error, but rather that it is not possible to tell if the two samples were drawn from separate populations. Applicant is directed to the attached text from the chapter by Freund et al. (2003. Statistical Methods, Second Edition, pp. 117 – 138), particularly the definition of "significance level" on p. 126 and the definition of "p value" on p. 133 for a more complete understanding of the way statistics are used in scientific papers. In the instant case, Rovin et al. used analyses of variance, followed by post-hoc Bonferroni-corrected pairwise comparisons (see p. 1295, second column "Statistical Analysis") rather than the standard argued by applicant. The teachings of Rovin indicate that this assay has so much variability that even if a 21% difference is detected when 12 experimental and 12 control samples are provided (see legend for Figure 2 which indicates that "[e]ach point represents the mean of at least 3 individual experiments done in quadruplicate").

On p. 13 of the remarks, applicant points out that Rovin found an 18% increase was significant. Applicant again asserts his own definition of significance (p. 13, second complete paragraph) which contradicts that provided on p. 1295 of Rovin. It is noted that this level is still greater than the changes reported in the specification, where only a 15% increase is considered important. However, taken with the finding that a 21% increase in this assay is not significant, this finding further supports the examiner's point that knowing the variability associated with the measurements is crucial to determining whether or not the artisan will conclude that samples are drawn from different populations. In the instant case, the specification does not disclose the variability in the sample, so a skilled artisan would not reasonably conclude that PRO4380

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induces mesangial cell proliferation. The basis for questioning the utility of the instantly-claimed product provided herein is based on sound scientific analysis. While applicant's arguments have been considered they are not persuasive because no evidence has been provided to substantiate the assertion that the 15% change observed is an appropriate cutoff point. Thus the rejection under 35 USC § 101 stands.

9. Claims 22 – 30, 32 – 35, and 38 – 41 are also rejected under 35 U.S.C. 112, first paragraph. Specifically, since the claimed invention is not supported by either a specific and substantial asserted utility or a well established utility for the reasons set forth above, one skilled in the art clearly would not know how to use the claimed invention.

10. Even if enablement were found for PRO4380, enablement would not be commensurate in scope with claims 22 – 30, 32 – 35, and 38 - 41, because the specification does not reasonably provide enablement for polynucleotides less than 100% identical to SEQ ID NO:56, or for polynucleotides less than 100% identical to those that encode SEQ ID NO:57, wherein the encoded polypeptides have the ability to induce mesangial cell proliferation. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

Applicant is directed to paragraph number 8 for a more complete discussion of why claims to SEQ ID NO:57 are not deemed to be able to induce mesangial cell proliferation. In summary, applicant has shown that in a single trial, SEQ ID NO:57 induced a 15% increase in the number of mesangial cells. Applicant did not disclose the variability in this system, and Rovin teaches that the variability in this assay is so high that even a 21% change, calculated as the difference between the average of 12 experimental and 12 control samples, is not significant. Freund teaches that it is not proper to conclude that samples are drawn from separate populations when the test statistic exceeds the significance level. In the instant case, the assay performed by Rovin appears to be identical to that performed by applicant. Both used mammalian mesangial kidney cells, and even used the same detection reagent from Promega. The teachings of Rovin indicate that knowing the variability level of the assay is crucial to making conclusions as to whether or not the differences are significant. Because applicant's results fall below both the lowest level shown by Rovin to be significant, and below a level shown by Rovin to be non-significant, a skilled artisan would not conclude that SEQ ID NO:57 increases mesangial cell proliferation in the absence of providing statistically significant results.

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Instead the artisan would conclude that the ability of SEQ ID NO:57 to induce proliferation is no different than that of a control.

On p. 14, third complete paragraph of the remarks applicant refers to pp. 109 – 111 of the specification as providing guidance as to how to make the claimed variants. However this text does not indicate which regions of PRO4380 (i.e. SEQ ID NO:57), or of the nucleic acids encoding PRO4380 (i.e. SEQ ID NO:56) are to be retained and which regions can be altered. Applicant also refers to p. 113, line 21 of the specification as teaching how to isolate DNA that encodes PRO polypeptides generally. However isolation of DNA is not at issue here, what is at issue is whether the specification provides an enabling disclosure of how to make and use nucleic acid variants commensurate in scope with the claims without resorting to undue experimentation. In this case, the artisan would have to undertake a large amount of experimentation and there is no guidance in the specification as to which regions of SEQ ID NO:56 must be retained. Applicant also argues that pp. 114 – 126 of the specification teach uses for PRO generally. It is noted that pp. 114 – 119 line 3 disclose how to select and transform host cells, how to select and use vectors, how to detect gene amplification, and how to purify polypeptides. None of these appear to be patentable utilities, since the claimed nucleic acid and encoded protein are not useful. Page 119 – 124 disclose uses of PRO that are not specific to the instantly-claimed molecule (i.e. use as hybridization probe to detect itself, as antisense to prevent its own expression, or in the construction of transgenic or knockout animals). Furthermore the utilities asserted on these pages are not germane to the scope of enablement rejection, as the relevant issue is whether the claims are enabled over their full scope, not whether there is any conceivable use for the nucleic acid.

11. Claims 22 – 26, 35, and 38 – 41 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. This is a written description rejection.

Applicant argues that the legal standard for the written description requirement is whether the disclosure “reasonably conveys to the artisan that the inventor had possession at that time of the later claimed subject matter.” The examiner agrees with applicant’s definition. Applicant argues that the amendments to the claims, particularly the recitation “has the ability to induce mesangial cell proliferation” is sufficient to overcome the written description rejection.

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The examiner disagrees. The specification does not disclose any nucleic acids encoding polypeptide sequences at least 80, 85, 90, 95, or 99% identical to SEQ ID NO:57 which have the stated activity, with the sole exception of SEQ ID NO:56, the nucleic acid which encodes SEQ ID NO:57. The claims are drawn to genera of nucleic acids, but only a single member of the genus is disclosed. A genus claim may be supported by a representative number of species as set forth in *Regents of the University of California v Eli Lilly & Co*, 119F3d 1559, 1569, 43 USPQ2d 1398, 1406 (Fed. Cir. 1997), which states:

“To fulfill the written description requirement, a patent specification must describe an invention and do so in sufficient detail that one skilled in the art can clearly conclude that “the inventor invented the claimed invention”. Lockwood v. American Airlines, Inc., 107 F.3d 1565, 1572, 41 USPQ2d 1961, 1966 (1997); In re Gosteli, 872 F.2d 1008, 1012, 10 USPQ2d 1614, 1618 (Fed. Cir. 1980) (“[T]he description must clearly allow persons of ordinary skill in the art to recognize that [the inventor] invented what is claimed.”) Thus, an applicant complies with the written description requirement “by describing the invention, with all its claimed limitations, not that which makes it obvious,” and by using “such descriptive means as words, structures, figures, diagrams, formulas, etc., that set forth the claimed invention.” Lockwood, 107 F.3d 1565, 1572, 41 USPQ2d at 1966.

An adequate written description of a polypeptide, “requires a precise definition, such as by structure, formula, chemical name, or physical properties,” not a mere wish or plan for obtaining the claimed chemical invention. Fiers v. Revel, 984 F.2d 1164, 1171, 25 USPQ2d 1601, 1606 (Fed. Cir. 1993): Accordingly, “an adequate written description of a DNA requires more than a mere statement that it is part of the invention and reference to a potential method for isolating it; what is required is a description of the DNA itself.” Id at 1170, 25 USPQ2d at 1606.” While the preceding quotation is drawn to DNA, the same logic applies to claims drawn to proteins and their variants.

Furthermore applicant has not shown which regions of the protein are important for the claimed activity. A description of a genus of proteins may be achieved by means of a recitation of a representative number of proteins, defined by amino acid sequence, falling within the scope of the genus, or of a recitation of structural features common to the genus, which features constitute a substantial portion of the genus. The instant specification discloses, however, a single isolated nucleic acid sequence SEQ ID NO:56 and a single polypeptide sequence SEQ ID NO:57. The additional limitation of requiring that the protein variants have the ability to

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induce mesangial cell proliferation fails to meet the written description requirement because it does not direct the artisan to those members of the claimed genera that meet the requirement. Furthermore the assay described has not been evidenced as providing either statistically or non-statistically related results that show conserved functions of the claimed members of the genera. Thus claims 22 – 26, 35, and 38 – 41 stand rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement.

Claim Rejections - 35 USC § 102

12. Claims 22 – 27, 29, 35, and 38 – 41 are rejected under 35 U.S.C. 102(a) as being anticipated by Ruben. The paragraph spanning pp. 13 – 14 of the previous office action indicate that Ruben teaches a sequence 95.3% identical to SEQ ID NO:56 as well as vectors and host cells comprising same. As set forth on p. 14 of the previous office action, the sequence from Ruben is identical to applicant's SEQ ID NO:56 from nucleotides 243 – 2134, including the entire region encoding PRO4380 without its signal peptide

On p. 17 of the remarks, applicant argues that the declaration of Goddard et al. is sufficient to overcome the rejections of claims 22 – 27, 29, 31, and 35 - 41. Applicant's arguments have been considered but are not persuasive. The declaration provides evidence that applicant was in possession of SEQ ID NO:56 and the protein encoded by it, SEQ ID NO:57, before the publication by Ruben. The declaration by Goddard et al. is not sufficient to overcome the rejection of claims 22 – 26 and 38 – 41. The declaration states that applicant was in possession of SEQ ID NO:56, but claims 22 – 26 are drawn to isolated nucleic acids at least 80, 85, 90, 95, or 99% identical to SEQ ID NO:56. The declaration does not provide evidence that applicant was in possession of any sequence less than 100% identical to SEQ ID NO:56 prior to the Ruben publication, nor does it provide evidence that applicant was in possession of a nucleic acid which hybridizes to SEQ ID NO:56 under the conditions recited in claim 35. Furthermore the declaration does not provide evidence that applicant was in possession of either SEQ ID NO:57 lacking its signal peptide or a nucleic acid encoding this fragment. These appear in claim 27, part (b) and claim 29. As stated in the previous office action, Ruben also teaches vectors and host cells which comprise the nucleic acid, and thus anticipates claims 38 – 41 as well.

Rejections Necessitated by Amendment

Claim Rejections - 35 USC § 112

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13. Claims 22 – 27, 30, 35, and 38 – 41 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. This is a new matter rejection.

The claims have been amended to recite “wherein the extracellular domain is amino acids 293 – 507”. There is no disclosure of this region being the extracellular domain. Figure 26 of the specification indicates the location of a transmembrane domain, but there is no disclosure of which end of the protein is intracellular and which end is extracellular. Since there was not disclosure of which regions were intracellular or extracellular in the specification, drawings, or claims as originally filed, identification of such regions is deemed to be new matter.

New Rejections and Objections

Oath/Declaration

14. The oath or declaration is defective. A new oath or declaration in compliance with 37 CFR 1.67(a) identifying this application by application number and filing date is required. See MPEP §§ 602.01 and 602.02.

The oath or declaration is defective because:

Non-initialed and/or non-dated alterations have been made to the oath or declaration, particularly the changes to citizenship by Zhang and to address by Eaton. See 37 CFR 1.52(c).

Claim Rejections - 35 USC § 102

15. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless –

(e) the invention was described in (1) an application for patent, published under section 122(b), by another filed in the United States before the invention by the applicant for patent or (2) a patent granted on an application for patent by another filed in the United States before the invention by the applicant for patent, except that an international application filed under the treaty defined in section 351(a) shall have the effects for purposes of this subsection of an application filed in the United States only if the international application designated the United States and was published under Article 21(2) of such treaty in the English language.

16. Claims 22 – 27, 29, 30, 35, and 38 – 41 are rejected under 35 U.S.C. 102(e) as anticipated by (U.S. Patent application publication 2003/0100051, published 29 May 2003, filed

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10 September 2001, claiming priority to applications filed 28 June 2001, 10 November 1999, 6 May 1999, and claiming benefit of provisional applications filed 11 September 2000, 18 May 1998, and 12 May 1998).

Ruben et al. teach SEQ ID NOs:137, 139, and 242, each of which are 97.0% identical to applicant's SEQ ID NO:57 (see attached alignments). Ruben's sequences are identical to applicant's SEQ ID NO:57 from residues 16 – 507 (using applicant's residue numbers). Ruben also teaches that his invention includes isolated nucleic acids encoding each of the sequences, thereby meeting the limitations of claims 22 – 25, part (a) of each claim (see Ruben, Table 1 on pp. 94 – 97 for the identification of which nucleic acids encode which proteins). Applicant has identified residues 1 – 26 as the signal peptide (see Figure 26). Since the sequences from Ruben et al. are 100% identical to SEQ ID NO:57 starting at residue 16, the prior art sequence also meets the limitations of claims 27, 29, and 30, drawn to isolated nucleic acids encoding polypeptides comprising SEQ ID NO:57 lacking its associated signal sequence, independent of which end of the polypeptide is the extracellular domain. Similarly, the teachings of Ruben also anticipate claims 27, 30, and 35, which are drawn nucleic acids encoding polypeptides comprising the extracellular domain of SEQ ID NO:57. The prior art polypeptide sequence from Ruben is 100% identical to residues 16 – 507 of SEQ ID NO:57 (using applicant's residue numbers), and includes residues 293-507 which is identified as the extracellular domain in amended claim 22, for example. Since the polypeptides taught by Ruben are identical to those recited in claim 35, parts (b) and (c), the nucleic acids encoding them will inherently hybridize to applicant's nucleic acids under the recited conditions.

The claims, as amended, recite the functional limitation requiring that the polypeptide has the ability to induce mesangial cell proliferation; Ruben is silent as to this property. However, since the only differences between the prior art product and applicant's product is in the signal sequence which is cleaved off during protein processing, Ruben's polypeptides will inherently have the claimed properties. Ruben also teaches the nucleic acids in vectors, including those with sequences recognized by host cells (see Table 1 and pp. 120 - 121). Ruben teaches how to insert vectors into host cells and includes E. coli, yeast and CHO cells as preferred host cells (see p. 121, paragraphs 0830 – 0832).

It is acknowledged that applicant's declarations under 37 CFR 1.131 is sufficient to indicate that he was in possession of SEQ ID NO:56 prior to 18 November 1999. However the publication by Ruben cited herein claims benefit of provisional applications filed 12 May 1998

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and 18 May 1998. These are the same applications cited on the face of WO 99/58660, which was the basis of the rejection under 35 USC 102 (a) in the previous office action. Applicant is advised that the declarations filed both 13 June and 1 July 2005 have been considered, although it is not immediately obvious to the examiner how the two declarations differ, as they appear to be duplicates.

Conclusion

17. No claim is allowed.

18. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Daniel Kolker whose telephone number is (571) 272-3181. The examiner can normally be reached on Mon - Fri 8:30AM - 5:00PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Janet Andres can be reached on (571) 272-0867. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Daniel E. Kolker, Ph.D.

September 12, 2005



SHARON TURNER, Ph.D.
PRIMARY EXAMINER

9-12-05

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

Application No. 10/035,977
 Filing Date December 26, 2001
 First Named Inventor Audrey Goddard
 Art Unit 1646
 Examiner Kolker, Daniel E.
 Attorney Docket No. GNE.3030RIC10

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U.S. PATENT DOCUMENTS

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Regulation of mesangial cell proliferation

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Regulation of mesangial cell proliferation. Regardless of the source of injury, an imbalance in the control of mesangial cell proliferation appears to play a direct role in the degree of progressive renal injury and glomerulosclerosis. Some of the regulatory mechanisms include specific soluble or non-soluble extracellular factors and a complex array of receptor-mediated signals that control the progression of the cell cycle or cell death. Understanding these regulatory processes could lead to novel therapeutic strategies to alleviate or arrest proliferative glomerular disease.

In the adult organism, normally quiescent cells exhibit increased replication rates during tumorigenesis or in the tissue repair processes following injury. In the renal glomerulus, very different types of injury are able to induce local inflammatory reactions involving resident glomerular cells. Lesions may be immune mediated, infectious, toxic, mechanical, or of other etiologies. A prominent histopathological feature of many human and experimental glomerular inflammatory diseases is cellular hyperplasia in the mesangium, which is caused by proliferation of mesangial cells (MCs) and an influx of leukocytes (Fig. 1). In this article we discuss the pathophysiological relevance of increased MC proliferation in the context of glomerular disease progression and review some of the extracellular factors and molecular mechanisms controlling MC growth.

RELEVANCE OF INCREASED MESANGIAL CELL PROLIFERATION

Pathogenetic linkage of mesangial cell hyperplasia and glomerulosclerosis

Because proliferation of MCs in the normal adult kidney is tightly regulated with a growth rate of less than 1% [1], quiescent MCs can be assumed to interact with no or few mitogens and/or to be protected by either down-regulation of receptors or the presence of growth-inhibitory factors maintaining the low proliferative activity. Regardless of the injurious mechanism, an imbalance

in the control of MC proliferation appears to play an early and crucial role in the pathogenesis of progressive glomerular injury and glomerulosclerosis. In experimental models of nephritis, MC proliferation frequently precedes and is linked to the increase of extracellular matrix (ECM) in the mesangium and glomerulosclerosis [2, 3]. For example, mice transgenic for the SV40 T antigen, which has growth-promoting functions, develop MC proliferation followed by progressive sclerosis [4], and mice transgenic for growth hormone ultimately develop severe progressive mesangial sclerosis. They show a five-fold increase in the ³H-thymidine-labeling index of glomerular cells. Interestingly, the labeling index remained high at late time points in densely sclerotic glomeruli [2], indicating that increased MC turnover can be a significant feature associated with sclerosis, both at the onset and in later stages.

Moreover, measures that reduce cell proliferation in glomerular disease models, such as treatment with heparin [5], low-protein diet [6], or neutralizing antibodies to platelet-derived growth factor (PDGF) [7], have been shown to reduce ECM expansion and sclerotic changes. Further evidence for the role of uncontrolled MC proliferation in the pathogenesis of glomerulosclerosis was provided by work of Pippin et al, who studied the effects of the pharmacological cdk2 inhibitor roscovitine on disease progression in mesangioproliferative anti-Thy1.1 nephritis of the rat [8]. The application of the purine analogue roscovitine resulted in a significant reduction of MC proliferation and glomerular cellularity in nephritic animals. Notably, the decrease of MC replication was associated with a marked reduction of mesangial ECM accumulation and less deposition of collagen type IV, laminin, and fibronectin [8].

Reconstitutive mesangial cell proliferation in glomerular repair

Persistent MC hyperplasia caused by repetitive or continuous glomerular injury is thought to precede and lead to irreversible glomerular scarring and the loss of renal function. However, like in regular wound healing, the transient increase of MC proliferation presumably reflects a physiological response required for successful

Key words: glomerulosclerosis, hyperplasia, tumorigenesis, injury, inflammation, lesions.

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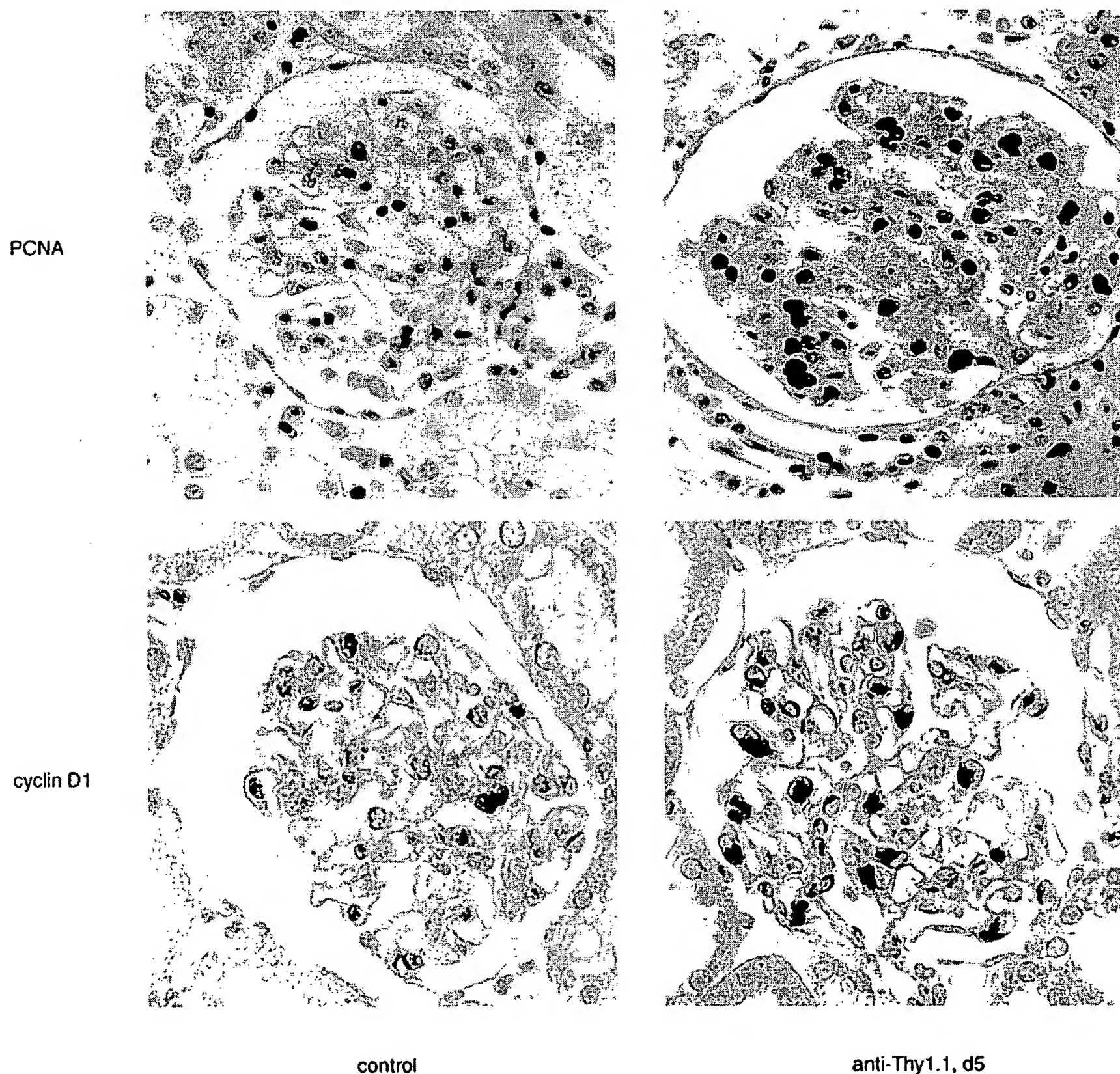


Fig. 1. Immunohistochemical detection of the proliferation marker, proliferating cell nuclear antigen (PCNA), and the G1 phase-regulatory protein cyclin D1 in rat kidney sections from normal control animal and five days after the induction of mesangioproliferative anti-Thy1.1 nephritis. PCNA-positive and cyclin D1-positive nuclei are stained black.

glomerular reconstitution and renal tissue repair. Evidence for this concept comes from observations in several types of human glomerular diseases, for example, poststreptococcal glomerulonephritis and some forms of lupus nephritis. These diseases show marked expansion of the mesangium because of MC hyperplasia, which may subsequently resolve completely once the inflammatory cause has subsided. In addition, experimental animal models of mesangiolytic injury characterized by a rapid

loss of MCs, such as anti-Thy1.1 nephritis [9] or Habu toxin glomerulopathy [10], exhibit marked proliferation of MCs leading to transient MC hyperplasia and matrix accumulation. If no repetitive insult to the mesangium is added in these animals, both diseases show spontaneous repair of the glomerular tufts within a few weeks, indicating that early and pronounced MC replication may indeed be part of the physiological reconstitutive response of glomeruli to injury, such as mesangiolysis. Thus, thera-

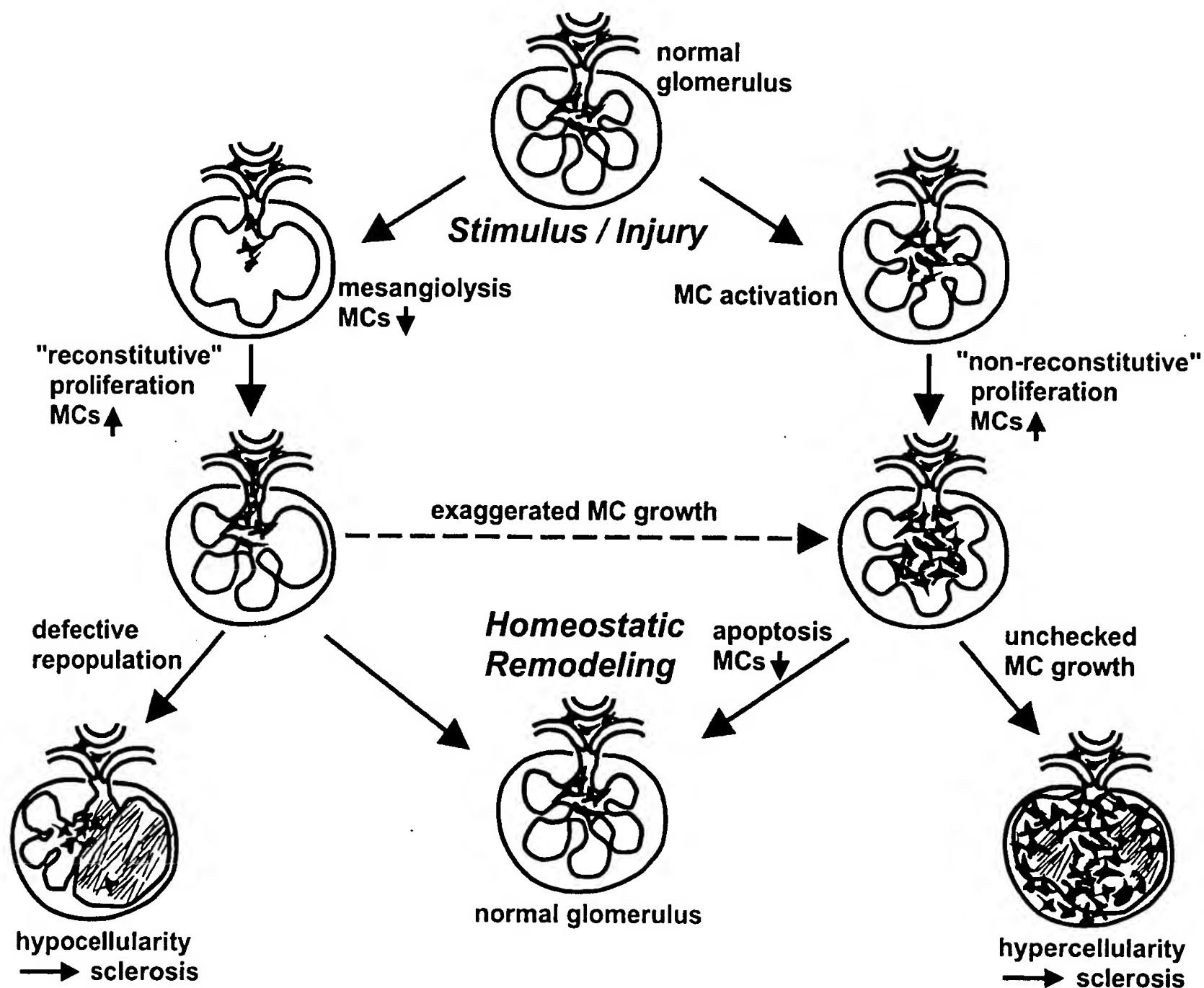


Fig. 2. Schematic illustration of two types of mesangial cell (MC) proliferation in glomerulonephritis and in homeostatic remodeling: "reconstitutive" proliferation of remaining MCs following mesangiolysis or "nonreconstitutive" MC replication after the activation of orthotopic MCs without prior cell loss. Defective homeostatic mechanisms are associated with extracellular matrix (ECM) accumulation and glomerular scarring.

peutical manipulations aiming to block MC mitogenesis could represent a double-edged sword. Although the available evidence suggests that control of overshooting MC replication is beneficial in reducing ECM accumulation, interference with reconstitutive proliferation of MCs could conceivably lead to incomplete cellular repopulation of the glomerular tuft and defective repair. Clearly, more precise markers than MC hyperplasia are needed to better characterize the dynamic nature of MC proliferation and its regulators in proliferative glomerulonephritis. This would also allow a more rational evaluation of the effects of growth-inhibitory manipulations in proliferative glomerular diseases following the loss of MCs and in chronic-progressive disease stages. Figure 2 schematically depicts two types of increased MC proliferation in response to glomerular injury. Conceptually, one

can distinguish between "reconstitutive" proliferation of the remaining MCs after mesangiolysis from "nonreconstitutive" or "orthotopic" MC replication in response to activation of intact MCs, for example, by soluble growth factors. However, it should be noted that *in vivo* these histological features may occur simultaneously, even within the same glomerulus. Currently, the methodology is insufficient to discern the nature of the proliferative processes in glomerulonephritis. This problem is aggravated in the evaluation of human glomerular diseases because of the fact that a regular kidney biopsy specimen yields but a limited number of tissue sections with rather few glomeruli. Currently, these limitations prevent thorough and meaningful studies of glomerular cell turnover in patients.

Apoptosis: A clearing mechanism balancing mesangial cell hyperplasia

When recovery of mesangioproliferative disease occurs, how is the resolution of hypercellularity achieved? Theoretically, resolution would require the reduction of the baseline regeneration or proliferation rate and/or increased removal or death of MCs. Recent research has examined cell apoptosis, a process that uses a controlled program to achieve cell death. In glomeruli, apoptotic bodies can be found in renal biopsies from patients with proliferative glomerulonephritis [11]. Baker et al and Shimizu et al demonstrated that MC apoptosis is a cell clearance mechanism counterbalancing MC division in self-limited anti-Thy1.1 glomerulonephritis, thereby contributing to the resolution of glomerular hypercellularity caused by experimentally induced MC proliferation in rats [12, 13]. In these experiments, mitotic and apoptotic cells were detected in the same glomerulus, suggesting that both processes can occur simultaneously. Although these studies imply a beneficial role of apoptosis as a mechanism leading to the reduction of MC hyperplasia and thereby contributing to the resolution of glomerular disease, other data indicate that excessive apoptosis can be harmful, leading to glomerular hypocellularity [14]. Again, these observations reflect the fact that glomerular MC numbers at any given stage during glomerular disease are determined by the rates of cell proliferation and loss. Clearly, homeostatic reconstitution of the glomerulus depends on a regulated balance between these two processes. The precise mechanisms controlling the glomerular cell turnover and net cell number in health and disease are currently incompletely understood.

MOLECULAR CONTROL OF MESANGIAL CELL CYCLE PROGRESSION

The division cycle of eukaryotic cells is controlled by a series of checkpoints and transitions in which temporal order is imposed by cyclin-dependent kinases (CDKs), acting in concert with their regulatory subunits, the cyclins (Fig. 3) [15]. Cyclin kinase inhibitors (CKIs), such as p21^{Waf-1}, p27^{Kip1}, and members of the INK4 family of CDK inhibitors, negatively regulate the cell cycle by inhibiting the formation or activation of cyclin-CDK complexes [16]. In the past few years, considerable advances have been made in this field of research, and nephrologists are applying this knowledge to elucidate the molecular control of cell cycle progression of renal cells, including MCs. New findings on molecular mechanisms of cell cycle control were presented at this symposium and are the subject of recent reviews [16–19].

The rat anti-Thy1.1 model of mesangioproliferative glomerulonephritis has been used to study the *in vivo* expression of cell cycle regulators in glomerular cells. Shankland et al showed that the onset of MC replication

in this model is associated with significant and transient increases of cyclin A and cdk2 protein [20]. Moreover, the increase of cdk2 protein expression in the course of nephritis correlated with an increase of cdk2 activity, as measured by histone H1 kinase assay in isolated glomeruli. In normal rat glomeruli, the expression of the CKI p27^{Kip1} was high, whereas the levels of p21^{Waf-1} were low. The onset of MC proliferation in the anti-Thy1.1 model was associated with a reduction of p27^{Kip1} levels. The resolution of MC proliferation was associated with a return to baseline levels for p27^{Kip1}, whereas the expression for p21^{Waf-1} further increased and remained elevated following the resolution of proliferation [20]. The relevance of the sustained p21^{Waf-1} was not fully explained, but one could speculate that p21^{Waf-1} has a functional role in the long-term resolution of mesangioproliferative disease. Currently, however, this interpretation is unproven.

Indeed, recent data have shown that p21^{Waf-1} has divergent functions. The CKI p21^{Waf-1} can act as an assembly factor of cdk4/cyclin D complexes or as an inhibitor of CDK/cyclin complexes [16, 21]. We have recently examined how soluble regulators of MC growth affect p21^{Waf-1} expression in cultured MCs. We found that PDGF causes a marked increase of p21^{Waf-1} protein in MCs [22]. The induction of p21^{Waf-1} protein by PDGF was also reported in p53-deficient as well as in normal mouse fibroblasts, supporting the interpretation of p53-independent up-regulation of p21^{Waf-1} by PDGF [23]. In other cell types, the growth-inhibitory cytokine transforming growth factor- β 1 (TGF- β 1) was found to enhance p21^{Waf-1} expression by p53-independent mechanisms [24]. However, our experiments showed that in cultured rat MCs, TGF- β 1 led to a slight reduction of the p21^{Waf-1} expression, which was induced by PDGF [22], whereas it strongly stimulated p27^{Kip1}. These divergent findings indicate that the nature of TGF- β 1-mediated effects on cell cycle regulatory proteins differs considerably between cell types.

In our studies, we observed that increased nuclear abundance of cyclin D1 protein in MCs precedes the peak of MC hypercellularity in anti-Thy1.1 glomerulonephritis (Fig. 1), suggesting its involvement in the development of mesangioproliferative disease [22]. To further examine the relevance of the *in vivo* finding of enhanced cyclin D1 expression for MC hyperplasia, we studied the effects of soluble regulators of MC growth on cyclin D1 expression *in vitro*. In growth-arrested rat MCs in culture, mitogenic stimulation with serum or PDGF led to a rapid increase of cyclin D1 protein expression. TGF- β 1 inhibited PDGF-related induction of cyclin D1 protein. Because cyclin D1 is a key regulator of early G1-phase progression and adenovirus-mediated overexpression of cyclin D1 in MCs increased MC mitogenesis [25], we examined whether the reduction of cyclin D1 protein abundance by transfection of antisense oligonu-

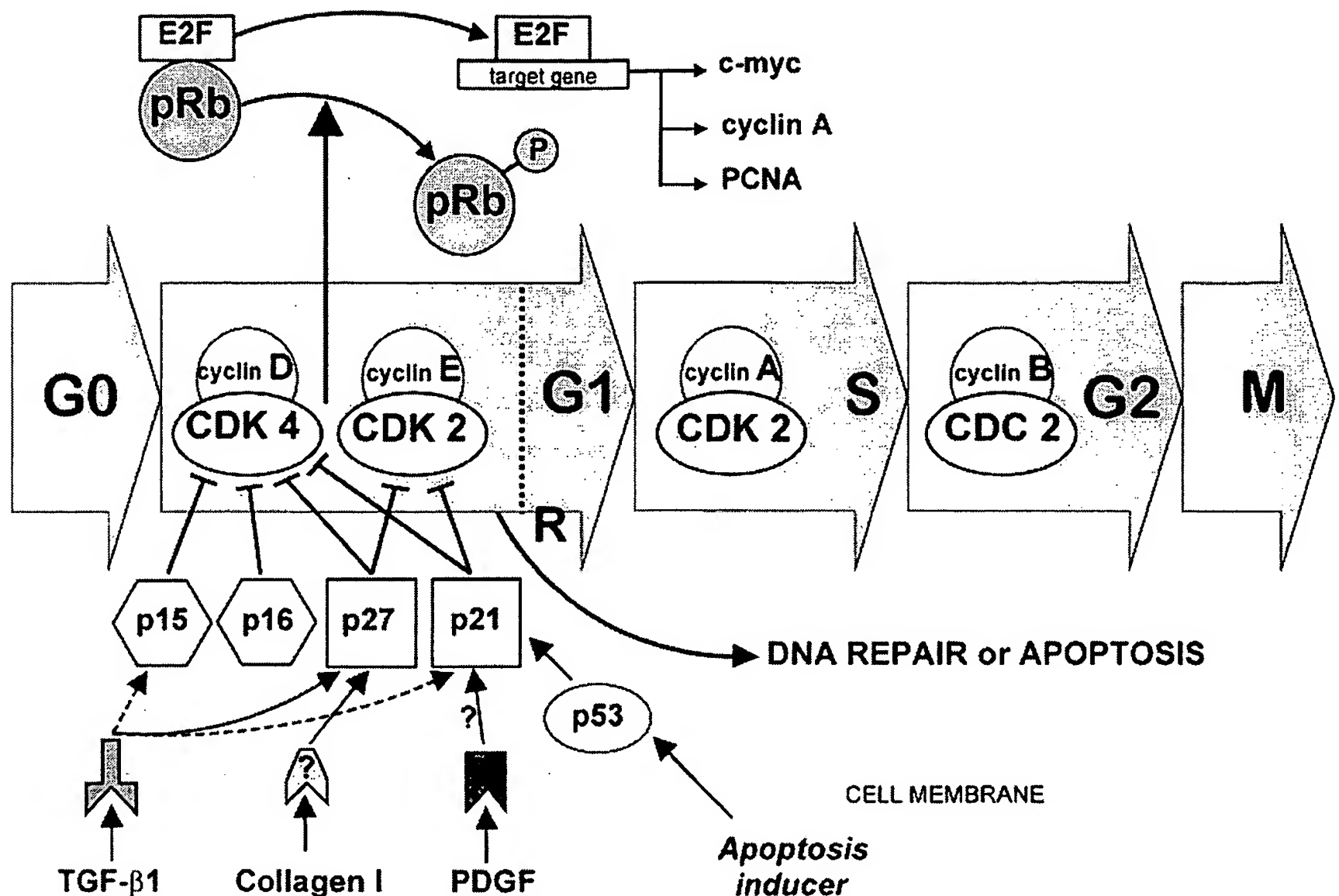


Fig. 3. Schematic diagram of the signaling pathway that mediates cell cycle progression through the G1 into the S phase. Cell cycle progression is controlled by cyclin-dependent kinases (CDK) and their regulatory subunits, the cyclins. Nuclear kinase inhibitors (for example, p15, p16, p21, and p27) modulate the enzymatic activity of cyclin/CDK complexes. External (for example, TGF- β , PDGF, collagen I) or internal regulators (for example, p53) influence the activity and/or expression of these inhibitors. The transcription factor E2F is repressed by its binding to the underphosphorylated form of the retinoblastoma gene product (pRb). Phosphorylation of pRb activates E2F, which induces the transcription of target genes essential for G1 and S phase progression. The abortion of G1 phase progression may result in apoptosis or allow for DNA repair. The restriction point (R) in the late G1 phase is where cells commit to completing the cell cycle.

cleotides (ODNs) is sufficient to inhibit cdk4 kinase activity and MC proliferation. Several studies successfully employed transfection of antisense ODNs to examine the roles of MC growth regulators and to block MC replication. In cultured MCs, proliferation could be inhibited by using antisense ODNs against the early growth response gene Egr-1 [26] or other cell cycle-associated proteins such as PCNA or Ki-67 [27]. We observed that antisense ODNs against cyclin D1 reduced the serum- or PDGF-induced protein expression of cyclin D1 to 48% or 10% of control levels, respectively [22]. These inhibitory effects correlated with diminished cdk4 activity. Subsequently, the MC proliferation caused by serum or PDGF was markedly inhibited by antisense ODNs against cyclin D1, as measured by [3 H]-thymidine uptake and MC counts. Antisense ODNs against cyclin D1 have also been shown to diminish the increase of DNA synthesis of rat MCs induced by endothelin-1 [28]. Thus, strate-

gies aiming to reduce cyclin D1 expression, for example, by transfer of antisense ODNs or by specific cyclin antagonists, might represent an effective means to inhibit MC proliferation *in vivo*.

Other studies investigated whether or not MC replication is reduced by suppressing the activity of transcription factor E2F. E2F controls the expression of several genes involved in cell cycle progression, including c-myc, c-myb, proliferating cell nuclear antigen (PCNA), and cdk2 kinase [19]. In order to inhibit E2F, decoy ODNs that contained the consensus E2F binding site sequence as a competitive inhibitor were transfected. Transfection of E2F decoy ODNs effectively reduced the proliferation of serum-stimulated MCs *in vitro* [29]. Moreover, an *in vivo* transfection of E2F decoy ODNs into rat kidneys 36 hours after the induction of anti-Thy1.1 suppressed MC proliferation by 71% [30]. These results correlated with reduced glomerular expression of PCNA [30], indicating

that decoy ODNs can inhibit the activity of E2F and may have the potential to treat proliferative forms of glomerulonephritis.

TRANSFORMING GROWTH FACTOR- β : AN AUTOCRINE INHIBITOR OF MESANGIAL CELL REPLICATION

In vitro and *in vivo* studies have identified a multitude of extracellular factors that stimulate or inhibit MC proliferation, including various soluble cytokines, autacoids, hormones, as well as insoluble ECM molecules. Many of these ligands are produced by MCs and possess autocrine growth-modulating activities [31]. Although numerous reports exist on promitogenic factors and signaling pathways in MCs, less is known about antimitogenic mechanisms that are able to block MC proliferation and might be involved in the maintenance of MC quiescence in the normal kidney and/or in the resolution of mesangioproliferative disease. TGF- β is a potent endogenous growth-inhibitory factor with the capacity to counterbalance MC mitogenesis; it is secreted by MCs and can act in an autocrine fashion [32]. Multiple studies of MCs in culture have confirmed the antimitogenic and ECM-promoting action of TGF- β [32–35]. Furthermore, in studies of experimental glomerular disease, TGF- β has been found to induce both beneficial, antimitogenic, anti-inflammatory effects [36] and harmful fibrogenesis leading to glomerular scarring [37–39]. Data from Kitamura et al demonstrated that mesangial overexpression of active TGF- β 1 by the use of MCs as a vector for gene delivery to the renal glomerulus reduced increased glomerular mitogenesis in anti-Thy1.1 nephritis, implying that TGF- β 1 acts as a suppressor of glomerular inflammation [36].

We have investigated nuclear regulatory events induced by TGF- β 1 in MCs [35]. TGF- β 1 abrogated mitogenesis of cultured MCs stimulated by PDGF, epidermal growth factor (EGF), serotonin, endothelin-1, or basic fibroblast growth factor (bFGF). By FACS analysis, we observed that the incubation of cultured MCs with TGF- β 1 blocks the progression of the MC cycle in the G1 phase of the cell cycle. One of the molecular mechanisms used by TGF- β 1 to arrest MCs in G1 phase was the significantly diminished activation of cyclin E/cdk2-complexes induced by PDGF. Subsequently, the decrease of cyclin E/cdk2 activity was reflected by less phosphorylation of the retinoblastoma tumor suppressor (pRb), a negative cell cycle regulator [35]. Deactivation of pRb via phosphorylation by G1 phase CDKs is a required step for successful G1-phase progression and S-phase entry [40]. Furthermore, in immunoprecipitation experiments we found that the presence of TGF- β 1 prevented the release of the CKI p27^{Kip1} from cyclin E/cdk2 complexes in the presence of soluble mitogens (unpublished results). This appears to be one molecular mechanism

by which cdk2 activity is negatively regulated by TGF- β 1 in MCs.

Transforming growth factor- β 1 has also been reported to cause hypertrophy of renal cells, including renal epithelial cells [41] and MCs [42, 43]. Work by Wolf et al has identified the CKI p27^{Kip1} as an important mediator of high-glucose-induced hypertrophy of MCs [44, 45]. Medium containing high D-glucose (450 mg/dl) stimulated the expression of p27^{Kip1} protein in cultured MCs. This induction of p27^{Kip1} involved activation of protein kinase C and was at least partly dependent on autoinduction of TGF- β in MCs [45]. Moreover, studies in the db/db mouse model of diabetes type II revealed an increase of p27^{Kip1} protein abundance in glomeruli of diabetic animals, which apparently was because of hyperglycemia [46]. With regard to MC growth control in glomerular disease, the available data indicate that TGF- β not only mediates the deposition of ECM but also functions as a potent autocrine inhibitor of G1-phase progression. Dependent on the type and phase of glomerular disease, the effects of TGF- β could therefore contribute to the resolution of hypercellular disease or lead to MC hypertrophy, a pathologic phenotype associated with expansion of the mesangium and, possibly, MC activation.

MODULATION OF MESANGIAL CELL GROWTH BY EXTRACELLULAR MATRIX

The ECM represents a very heterogeneous source of molecular information that profoundly regulates cell behavior. Changes in the quantity and composition of the mesangial ECM can directly affect MC biology; in turn, changes in the MC activation status may result in altered ECM synthesis. Under normal conditions, mesangial ECM compounds are synthesized and degraded in a balanced manner because the integrity of glomerular matrices is strictly maintained throughout adult life. However, the loss of coordinated regulation with increase in mesangial ECM deposition occurs in the development of glomerulosclerosis, a common feature of many forms of chronic inflammatory glomerular disease.

Studies in the last decade have revealed that in addition to soluble regulator molecules, nonsoluble ECM compounds can also influence MC growth. *In vitro*, freshly seeded MCs do not only plate with much higher efficiency on collagen IV and fibronectin compared with plastic, but also show greater replication activity [47]. Thrombospondin-1, an antiadhesive matricellular glycoprotein that promotes cell rounding and division in a variety of cell types, also increases MC proliferation. This effect might be mediated in part by up-regulation of epidermal growth factor and PDGF secretion, which, in turn, can increase MC proliferation in an autocrine manner [48]. Several investigators observed dose-dependent inhibition of MC proliferation by heparan sulfate

[49, 50]. Furthermore, several proteoglycans are able to bind and sequester various cytokines, for example, TGF- β and bFGF. Local storage of such factors could represent an important regulatory function of glomerular proteoglycans. Morita et al reported that heparan sulfate proteoglycans show an enrichment of bFGF-binding domains in fibrotic lesions of the peritubular interstitium [51]. Taken together, these results support the notion that ECM molecules bind and sequester growth factors, possibly providing a local "reservoir" of regulatory proteins and presenting them to adjacent cells in a biologically more active form. This concept, however, needs experimental proof.

Several studies performed with fibroblasts have explored the molecular basis of anchorage-dependent cell growth on the level of cell cycle regulators. Zhu et al demonstrated that cell adhesion induces cyclin D1 mRNA and is required for cyclin E/cdk2 activation and subsequent phosphorylation of pRb, an effect that is possibly mediated by down-regulation of the nuclear CDK inhibitor proteins p21^{Waf-1} and p27^{Kip1} [52]. Similarly, Fang et al showed that the cyclin E/cdk2 complex is activated in attached fibroblasts, but not in fibroblasts maintained in suspension [53]. These data establish a cell cycle basis to explain the combined requirement of both growth factors and ECM for anchorage-dependent growth of cells that are in transit through the G1 phase and entry into the S phase.

In addition to the need of mere cell anchorage for proliferation, the pattern of expressed adhesion molecules may also influence cell cycle progression. The expression of integrin $\beta 1C$, an alternatively spliced variant of $\beta 1$ integrin, has been described to inhibit growth by blocking cell cycle progression in the late G1 phase in fibroblasts [54] and epithelial cells [55]. Recently, integrin $\beta 1C$ was identified as an upstream regulator of the CKI p27^{Kip1}: overexpression of integrin $\beta 1C$ was associated with induction of p27^{Kip1} protein levels, inhibition of cyclin A-dependent kinase activity, and increased association of p27^{Kip1} with cyclin A [56]. Giancotti et al have examined mitogenic signaling mechanisms mediated by integrin receptors in endothelial cells and fibroblasts and could identify pathways specific for subgroups of integrins (discussed in F. Giancotti's abstract in this Symposium issue). Although most integrins are capable of activating focal adhesion kinase upon attachment to ECM, $\alpha_1\beta_1$, $\alpha_5\beta_1$, $\alpha_v\beta_3$, and $\alpha_6\beta_4$ integrins are coupled to the ras-ERK pathway via recruitment of the adaptor protein shc [57–59]. The shc adaptor links various tyrosine kinases to ras by recruiting the Grb2/SOS complex to the cell membrane [60]. In endothelial cells and keratinocytes, ligation of integrins linked to shc enabled the progression through the G1 phase in response to soluble mitogens. In contrast, ligation of other integrins resulted in growth arrest, even in the presence of soluble mitogens [58, 61].

The results of these studies indicate a direct growth-promoting function of integrin-dependent shc signaling in anchorage-dependent growth. Recently, caveolin-1 was identified as a membrane adaptor protein that links the integrin α chain to the tyrosine kinase c-fyn, which, in turn, activates and binds shc [59]. Results of our current studies have shown that these signaling molecules are also operative and regulated in cultured MCs (unpublished observations). However, the precise nuclear signaling events that mediate ECM effects to control quiescence or proliferation of MCs remain to be clarified.

Stimulated by work performed in vascular smooth muscle cells [62], we have recently tested the hypothesis that type I collagen inhibits MC proliferation by modulating cell cycle regulatory proteins of MCs in culture [63]. Although type I collagen is not expressed in glomeruli of healthy kidneys, type I collagen accumulates in the mesangial ECM in certain forms of glomerulonephritis. In the turnover of ECM, type I collagen is constantly degraded and structurally modified, for example, by proteolytic enzymes. MCs have been shown to bind to native type I collagen predominantly via $\alpha_1\beta_1$ and $\alpha_2\beta_1$ integrins. As an *in vitro* system to study the effects of structurally altered ECM molecules, we examined how two structurally distinct forms of type I collagen, monomer versus polymerized fibrils, affect proliferation and expression of G1-phase regulators in MCs, using two-dimensional cell culture. The adhesion of MCs to monomer or polymerized collagen was similarly effective. However, in contrast to control substratum of plastic or monomer collagen, polymerized collagen completely prevented the serum-induced increase of DNA synthesis and MC replication. The inhibitory effect of polymerized type I collagen was characterized by the rapid down-regulation of cyclins D1 and E and the lack of serum-mediated suppression of the CKI p27^{Kip1} [63]. We speculate that polymerized type I collagen fibrils specifically regulate early $\alpha_1\beta_1$ or $\alpha_2\beta_1$ integrin signaling, which leads to inhibition of MC proliferation. In cultured MCs, polymerized type I collagen also diminished c-fos induction and the activation of various kinases involved in intracellular signaling events [64]. This study did not examine whether the ECM-driven cellular effects are regulated by integrin-mediated signals.

CONCLUSION

Taken together, the control of quiescence, proliferation, and survival of MCs greatly affects glomerular structure and function in normal or diseased kidneys. Some of the regulatory mechanisms that are operative in MCs in culture and *in vivo* have recently been clarified. They involve multiple specific soluble or nonsoluble extracellular factors, and a complex array of receptor-mediated signals that control cell cycle progression as well as

apoptosis. A better understanding of such regulatory processes is hoped to lead to more rational diagnostic as well as therapeutic approaches in patients with proliferative glomerular disease.

ACKNOWLEDGMENTS

Research data for this review were obtained with the support of the Deutsche Forschungsgemeinschaft, Bonn; Sonderforschungsbe-
reich 263/B5.

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significance

An analytical evaluation of the results of a comparative trial or survey. Data yielding a difference in outcome depending on treatment or environmental factors are considered statistically significant if various mathematical procedures indicate there is less than a one in twenty (five percent) chance that the same results would occur through random accident. (In statistical terms this is expressed as $p < .05$ -or, the p-value is less than 0.05.)

(09 Oct 1997)

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Statistical Methods



Second Edition

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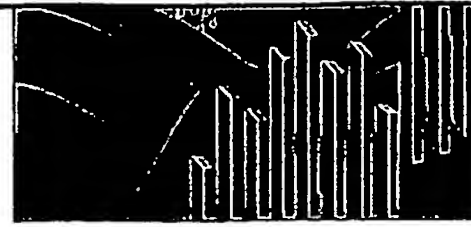


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Chapter 3



Principles of Inference

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EXAMPLE 3.1

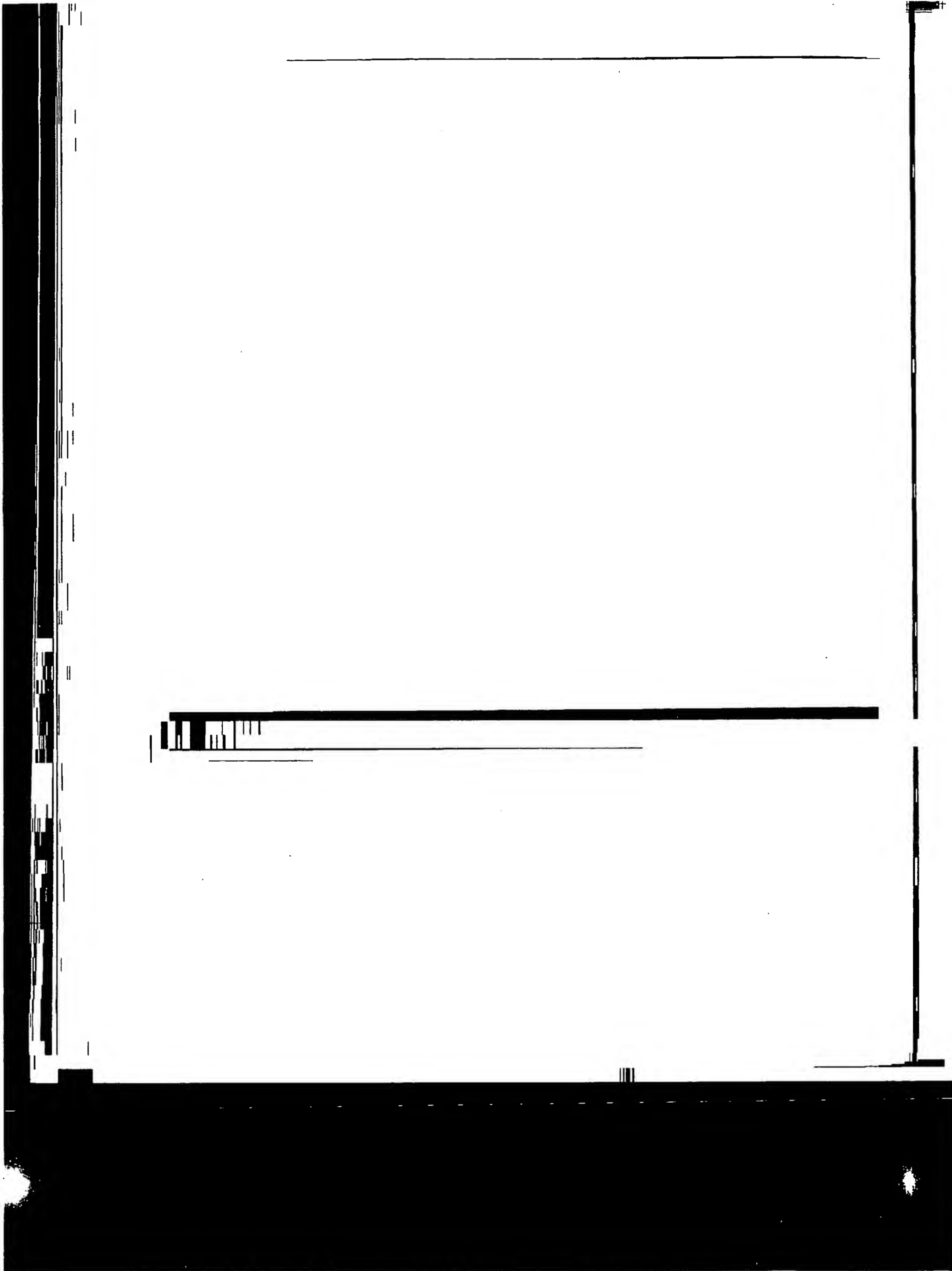
Is Office Rent More Expensive in Atlanta? A businessman in Atlanta is considering moving to Jacksonville, Florida, to reduce the office rental costs for his company. In the October 1990 issue of the *Jacksonville Journal*, the mean cost of leasing office space for all downtown buildings in Jacksonville was quoted as being \$12.61 per square foot with a standard deviation of \$4.50. To compare costs with those in Atlanta, the businessman sampled 36 office buildings in Atlanta and found a mean leasing cost of \$13.55 per square foot. Does this mean that leasing office space in Atlanta is really higher? Should the businessman consider moving to Jacksonville to save money on rent (assuming other factors equal)? This chapter presents methodology that can be used to help answer this question. This problem will be solved in Section 3.2. ■

3.1 Introduction

As we have repeatedly noted, one of the primary objectives of a statistical analysis is to use data from a sample to make inferences about the population from which the sample was drawn. In this chapter we present the basic procedures for making such inferences.

As we will see, the sampling distributions discussed in Chapter 2 play a pivotal role in statistical inference. Because inference on an unknown population parameter is usually based solely on a statistic computed from a single sample, we rely on these distributions to determine how reliable this inference is. That is, a statistical inference is composed of two parts:

1. a *statement* about the value of that parameter, and
2. a measure of the *reliability* of that statement, usually expressed as a probability.



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- A test of the effect of a diet pill on weight loss would be based on observed weight losses of a sample of healthy adults. If the test concludes the pill is effective, the manufacturer can safely advertise to that effect.
- To determine whether a teaching procedure enhances student performance, a sample of students would be tested before and after exposure to the procedure and the differences in test scores subjected to a statistical hypothesis test. If the test concludes that the method is not effective, it will not be used.

General Considerations

To illustrate the general principles of hypothesis testing, consider the following two simple examples:

EXAMPLE 3.2

There are two identically appearing bowls of jelly beans. Bowl 1 contains 60 red and 40 black jelly beans, and bowl 2 contains 40 red and 60 black jelly beans. Therefore, the proportion of red jelly beans, p , for the two bowls are

$$\text{Bowl 1: } p = 0.6$$

$$\text{Bowl 2: } p = 0.4.$$

One of the bowls is sitting on the table, but you do not know which one it is (you cannot see inside it). You suspect that it is bowl 2, but you are not sure. To test your hypothesis that bowl 2 is on the table you sample five jelly beans.¹ The data from this sample, specifically the number of red jelly beans, is the sample statistic that will be used to test the hypothesis that bowl 2 is on the table. That is, based on this sample, you will decide whether bowl 2 is the one on the table. ■

EXAMPLE 3.3

A company that packages salted peanuts in 8-oz. jars is interested in maintaining control on the amount of peanuts put in jars by one of its machines. Control is defined as averaging 8 oz. per jar and not consistently over- or underfilling the jars. To monitor this control, a sample of 16 jars is taken from the line at random time intervals and their contents weighed. The mean weight of peanuts in these 16 jars will be used to test the hypothesis that the machine is indeed working properly. If it is deemed not to be doing so, a costly adjustment will be needed.² ■

These two examples will be used to illustrate the procedures presented in this chapter.

¹To make the necessary probability calculations easier, you replace each jelly bean before selecting a new one; this is called sampling with replacement and allows the use of the binomial probability distribution presented in Section 2.3.

²Note the difference between this problem and Example 2.13, the control chart example. In this case, a decision to adjust the machine is to be made on one sample only, while in Example 2.13 it is made by an examination of its performance over time.



which is the specification for the machine to be functioning correctly. The alternative hypothesis is

$$H_1: \mu \neq 8,$$

which means the machine is malfunctioning. These statements also form a set of two exclusive and exhaustive hypotheses, even though the alternative hypothesis does not specify a single value as it did for Example 3.2.

Rules for Making Decisions

After stating the hypotheses we specify what sample results will lead to the rejection of the null hypothesis. Intuitively, sample results (summarized as sample statistics) that lead to rejection of the null hypothesis should reflect an apparent contradiction to the null hypothesis. In other words, if the sample statistics have values that are unlikely to occur if the null hypothesis is true, then we decide the null hypothesis is false. The statistical hypothesis testing procedure consists of defining sample results that appear to sufficiently contradict the null hypothesis to justify rejecting it.

In Section 2.5 we showed that a sampling distribution can be used to calculate the probability of getting values of a sample statistic from a given population. If we now define "unlikely" as some small probability, we can use the sampling distribution to determine a range of values of a sample statistic that is unlikely to occur if the null hypothesis is true. The occurrence of values in that range may then be considered grounds for rejecting that hypothesis. Statistical hypothesis testing consists of appropriately defining that region of values.

DEFINITION 3.3

The rejection region (also called the critical region) is the range of values of a sample statistic that will lead to rejection of the null hypothesis.

In Example 3.2, the null hypothesis specifies the bowl having the lower proportion of red jelly beans; hence observing a large proportion of red jelly beans would tend to contradict the null hypothesis. For now, we will arbitrarily decide that having a sample with all red jelly beans provides sufficient evidence to reject the null hypothesis. If we let Y be the number of red jelly beans, the rejection region is defined as $y = 5$.

In Example 3.3, any sample mean weight \bar{Y} not equal to 8 oz. would seem to contradict the null hypothesis. However, since some variation is expected, we would probably not want to reject the null hypothesis for values reasonably close to 8 oz. For the time being we will arbitrarily decide that a mean weight of below 7.9 or above 8.1 oz. is not "reasonably close," and we will therefore reject the null hypothesis if the mean weight of our sample occurs in this region. Thus, the rejection region for this example contains the values of $\bar{y} < 7.9$ or $\bar{y} > 8.1$.

If the value of the sample statistic falls in the rejection region, we know what decision to make. If it does not fall in the rejection region, we have a





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never know whether any error has been committed. The only way to avoid any chance of error is not to make a decision at all, hardly a satisfactory alternative.

Probabilities of Making Errors

If we assume that we have the results of a random sample, we can use the characteristics of sampling distributions presented in Chapter 2 to calculate the probabilities of making either a type I or type II error for any specified decision rule.

DEFINITION 3.6

α : denotes the probability of making a type I error
 β : denotes the probability of making a type II error

The ability to provide these probabilities is a key element in statistical inference, because they measure the reliability of our decisions. We will now show how to calculate these probabilities for our examples.

Calculating α for Example 3.2 The null hypothesis specifies that the probability of drawing a red jelly bean is 0.4 (bowl 2), and the null hypothesis is to be rejected with the occurrence of five red jelly beans. Then the probability of making a type I error is the probability of getting five red jelly beans in a sample of five from bowl 2. If we let Y be the number of red jelly beans in our sample of five, then

$$\alpha = P(Y = 5 \text{ when } p = 0.4).$$

The use of binomial probability distribution (Section 2.3) provides the result $\alpha = (0.4)^5 = 0.01024$. Thus the probability of incorrectly rejecting a true null hypothesis in this case is 0.01024; that is, there is approximately a 1 in 100 chance that bowl 2 will be mislabeled bowl 1 using the described decision rule.

Calculating α for Example 3.3 For this example, the null hypothesis was to be rejected if the mean weight was less than 7.9 or greater than 8.1 oz. If \bar{Y} is the sample mean weight of 16 jars, the probability of a type I error is

$$\alpha = P(\bar{Y} < 7.9 \text{ or } \bar{Y} > 8.1 \text{ when } \mu = 8).$$

Assume for now that we know³ that σ , the standard deviation of the population of weights, is 0.2 and that the distribution of weights is approximately normal. If the null hypothesis is true, the sampling distribution of the mean of 16 jars is normal with $\mu = 8$ and $\sigma = 0.2/\sqrt{16} = 0.05$ (see discussion on the normal distribution in Section 2.5). The probability of a type I error corresponds to the shaded area in Fig. 3.1.

³This is an assumption made here to simplify matters. In Chapter 4 we present the method required if we calculate the standard deviation from the sample data.

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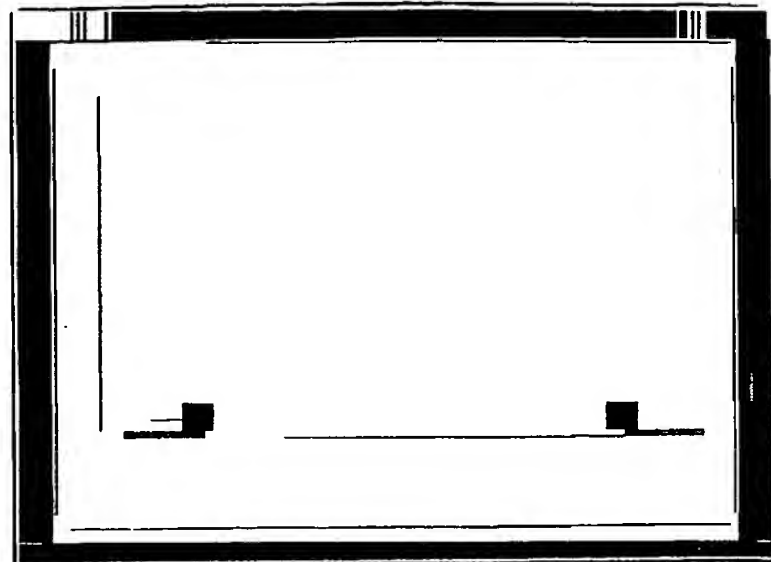
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Using the tables of the normal distribution we compute the area for each portion of the rejection region

$$P(\bar{Y} < 7.9) = P\left[Z < \frac{7.9 - 8}{(0.2/\sqrt{16})}\right] = P(Z < -2.0) = 0.0228$$

and

$$P(\bar{Y} > 8.1) = P\left(Z > \frac{8.1 - 8}{0.2/\sqrt{16}}\right) = P(Z > 2.0) = 0.0228.$$

Hence,

$$\alpha = 0.0228 + 0.0228 = 0.0456.$$

Thus the probability of adjusting the machine when it does not need it (using the described decision rule) is slightly less than 0.05 (or 5%).

Calculating β for Example 3.2 Having determined α for a specified decision rule, it is of interest to determine β . This probability can be readily calculated for Example 3.2. Recall that the type II error occurs if we fail to reject the null hypothesis when it is not true. For this example, this occurs if bowl 1 is on the table but we did not get the five red jelly beans required to reject the null hypothesis that bowl 2 is on the table. The probability of a type II error, which is denoted by β , is then the probability of getting four or fewer red jelly beans in a size-five sample from bowl 1. If we let Y be the number of red jelly beans in the sample, then

$$\beta = P(Y \leq 4 \text{ when } p = 0.6).$$

Using the probability rules from Section 2.2, we know that

$$P(Y \leq 4) + P(Y = 5) = 1.$$

Since $(Y = 5)$ is the complement of $(Y \leq 4)$,

$$P(Y \leq 4) = 1 - P(Y = 5).$$

Now

$$P(Y = 5) = (0.6)^5,$$

and therefore

$$\beta = 1 - (0.6)^5 = 1 - 0.07776 = 0.92224.$$

That is, the probability of making a type II error in Example 3.2 is over 92%. This value of β is unacceptably large. That is, if on the basis of this test we conclude that bowl 2 is on the table, the probability that we are wrong is 0.92!

Calculating β for Example 3.3 For Example 3.3, H_1 does not specify a single value for μ but instead includes all values of $\mu \neq 8$. Therefore, calculating the probability of the type II error requires that we examine the probability of the sample mean being outside the rejection region for every value of $\mu \neq 8$. These calculations and further discussion of β are presented later in this section where we discuss type II errors.

Choosing between α and β

The probability of making a type II error can be decreased by making rejection easier, which is accomplished by making the rejection region larger. For example, suppose we decide to reject H_0 if either four or five of the jelly beans are red. In this case,

$$\alpha = P(Y \geq 4 \text{ when } p = 0.4) = 0.087$$

and

$$\beta = P(Y < 4 \text{ when } p = 0.6) = 0.663.$$

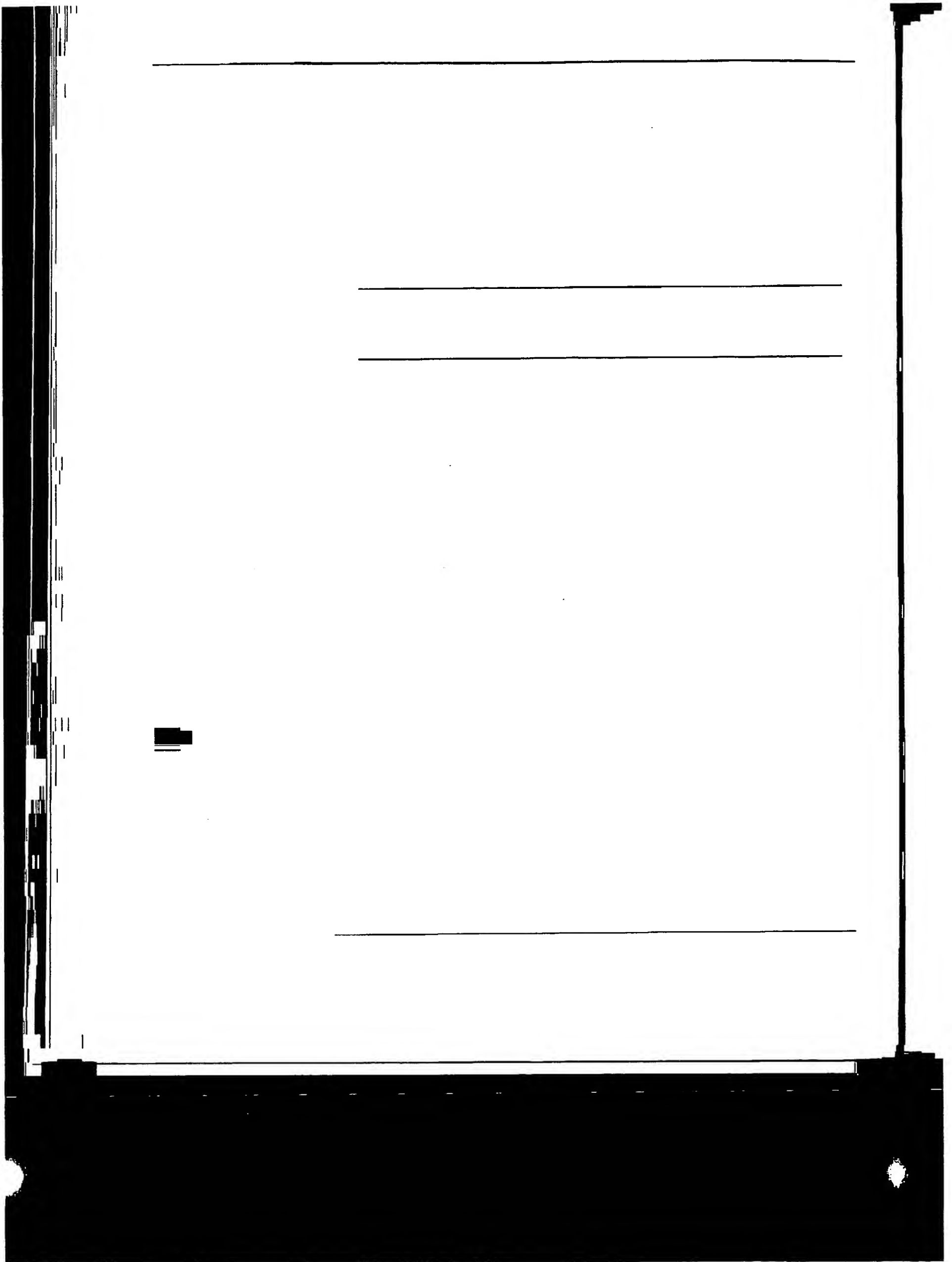
Note that by changing the rejection region we succeeded in lowering β but we increased α . This will always happen if the sample size is unchanged. In fact, if by changing the rejection region α becomes unacceptably large, no satisfactory testing procedure is available for a sample of five jelly beans, a condition that often occurs when sample sizes are small (see Section 3.4). This relationship between the two types of errors prevents us from constructing a hypothesis test that has a probability of 0 for either error. In fact, the only way to ensure that $\alpha = 0$ is to never reject a hypothesis, while to ensure that $\beta = 0$ the hypothesis should always be rejected, regardless of any sample results.

Five-Step Procedure for Hypothesis Testing

In the above presentation we have shown how to determine the probability of making a type I error for some arbitrarily chosen rejection region. The more frequently used method is to specify an acceptable maximum value for α and then delineate a rejection region for a sample statistic that satisfies this value. A hypothesis test can be formally summarized as a five-step process. Briefly these steps are as follows:

Step 1: Specify H_0 , H_1 , and an acceptable level of α .

Step 2: Define a sample-based test statistic and the rejection region for the specified H_0 .



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On the other hand, the alternative hypothesis describes conditions for which something will be done. It is the action or research hypothesis. In an experimental or research setting, the alternative hypothesis is that an established (status quo) hypothesis is to be replaced with a new one. Thus, the research hypothesis is the one we actually want to support, which is accomplished by rejecting the null hypothesis with a sufficiently low level of α such that it is unlikely that the new hypothesis will be erroneously pronounced as true.

In Example 3.2, we thought the bowl was 2 (the status quo), and would only change our mind if the sample showed significant evidence that we were wrong. In Example 3.3 the status quo is that the machine is performing correctly; hence the machine would be left alone unless the sample showed so many or so few peanuts so as to provide sufficient evidence to reject H_0 .

We can now see that it is quite important to specify an appropriate significance level. Because making the type I error is likely to have the more serious consequences, the value of α is usually chosen to be a relatively small number, and smaller in some cases than in others. That is, α must be selected so that an acceptable level of risk exists that the test will incorrectly reject the null hypothesis. Historically and traditionally, α has been chosen to have values of 0.10, 0.05, or 0.01, with 0.05 being most frequently used. These values are not sacred but do represent convenient numbers and allow the publication of statistical tables for use in hypothesis testing. We shall use these values often throughout the text. (See, however, the discussion of p values later in this section.)

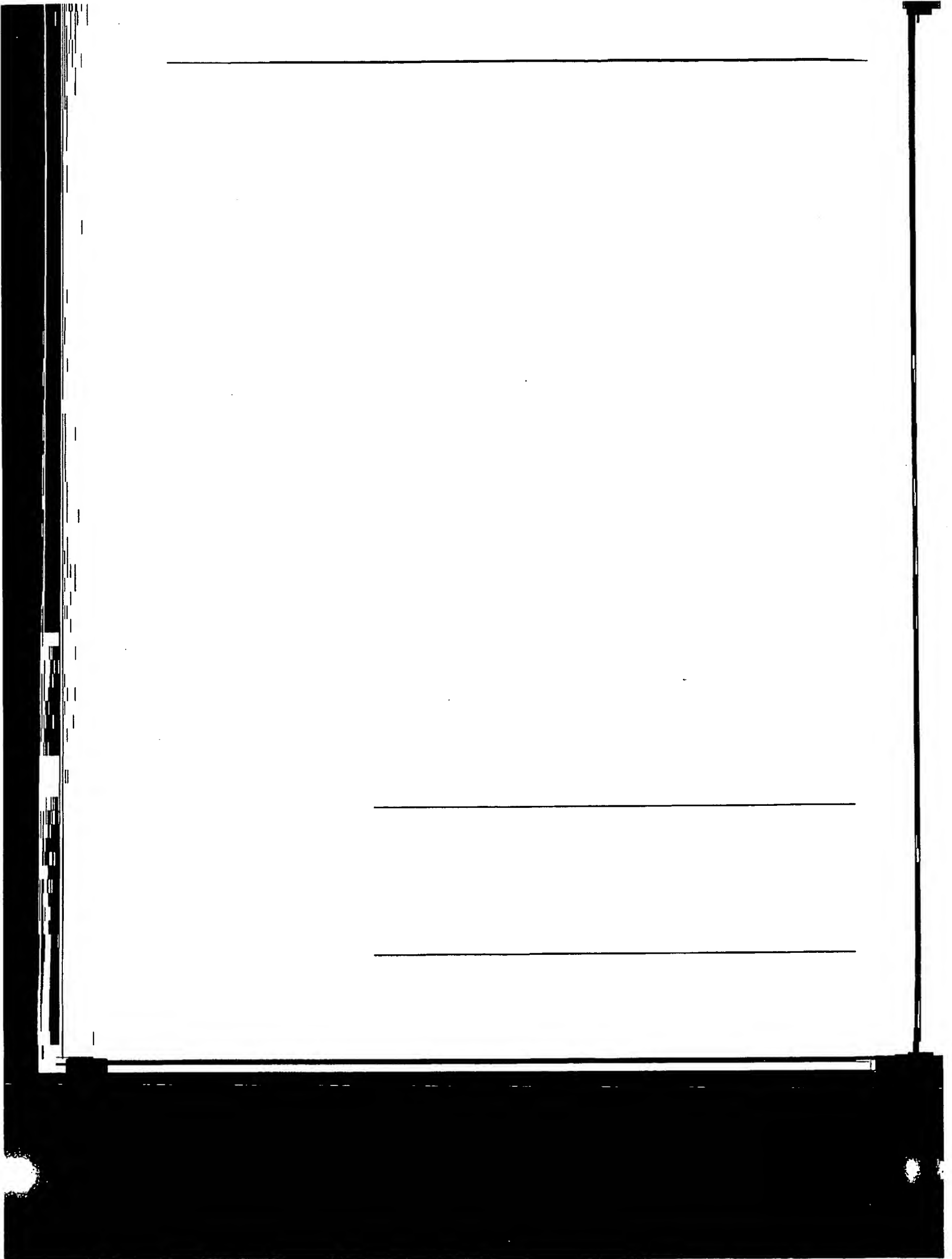
Choosing α

As we saw in Example 3.2, α and β are inversely related. Unless the sample size is increased, we can reduce α only at the price of increasing β . In Example 3.2 there was little difference in the consequences of a type I or type II error; hence, the hypothesis test would probably be designed to have approximately equal levels of α and β . In Example 3.3 making the type I error will cause a costly adjustment to be made to a properly working machine, while if the type II error is committed we do not adjust the machine when needed. This error also entails some cost such as wasted peanuts or unsatisfied customers. Unless the cost of adjusting the machine is extremely high, a reasonable choice here would be to use the "standard" value of 0.05.

Some examples of problems for which one or the other type of error is more serious include the following:

- Malnutrition among young children can have serious consequences. Assume that six-year-old children should average about 10 kg in weight to be considered normal. If a sample of children from a low-income neighborhood is to be tested⁵ for subnormal weight, we would probably use $H_0: \mu = 10$ kg

⁵An alternative hypothesis that specifies values in only one direction from the null hypothesis is called a one-sided or one-tailed alternative and requires some modifications in the testing procedure. One-tailed hypothesis tests are discussed later in this section.



DEFINITION 3.9

The rejection region comprises the values of the test statistic for which (1) the probability when the null hypothesis is true is less than or equal to the specified α and (2) probabilities when H_1 is true are greater than they are under H_0 .

In Step 2 we define the test statistic and the rejection region.

For Example 3.3 the appropriate test statistic is the sample mean. The sampling distribution of this statistic has already been used to show that the initially proposed rejection region of $\bar{y} < 7.9$ and $\bar{y} > 8.1$ produces a value of 0.0456 for α . If we had wanted α to be 0.05, this rejection region would appear to have been a very lucky guess! However, in most hypothesis tests it is necessary to specify α first and then use this value to delineate the rejection region. In the discussion of the significance level for Example 3.3 an appropriate level of α was chosen to be 0.05.

Remember, α is defined as

$$P(\bar{Y} \text{ falls in the rejection region when } H_0 \text{ is true}).$$

We define the rejection region by a set of boundary values, often called critical values, that are denoted by $C1$ and $C2$. The probability α is then defined as

$$P(\bar{Y} < C1 \text{ when } \mu = 8) + P(\bar{Y} > C2 \text{ when } \mu = 8).$$

We want to find values of $C1$ and $C2$ so that this probability is 0.05. This is obtained by finding the $C1$ and $C2$ that satisfy the expression

$$\alpha = P\left[Z < \frac{C1 - 8}{0.2/\sqrt{16}}\right] + P\left[Z > \frac{C2 - 8}{0.2/\sqrt{16}}\right] = 0.05,$$

where Z is the standard normal variable. Because of the symmetry of the normal distribution, exactly half of the rejection region is in each tail; hence,

$$P\left[Z < \frac{C1 - 8}{0.05}\right] = P\left[Z > \frac{C2 - 8}{0.05}\right] = 0.025.$$

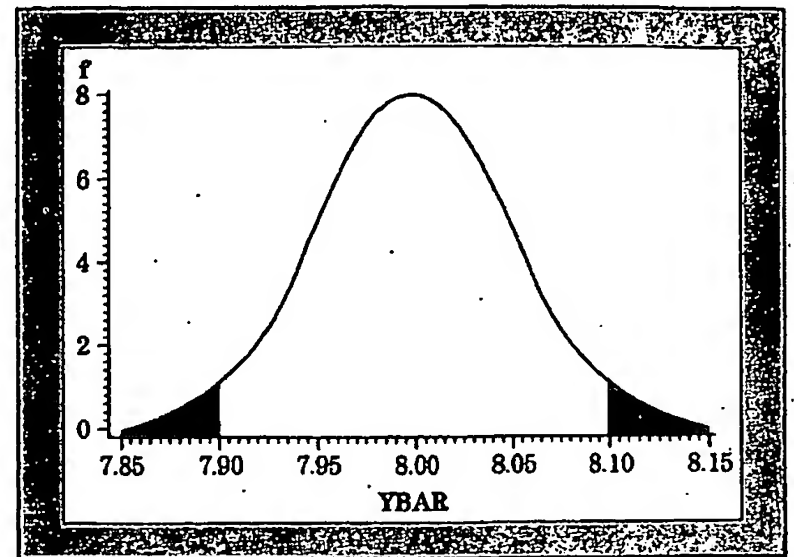
The values of $C1$ and $C2$ that satisfy this probability statement are found by using the standard normal table, where we find that the values of $z = -1.96$ and $z = +1.96$ satisfy our probability criteria. We use these values to solve for $C1$ and $C2$ in the equations $[(C1 - 8)/0.05] = -1.96$ and $[(C2 - 8)/0.05] = 1.96$. The solution yields $C1 = 7.902$ and $C2 = 8.098$; hence, the rejection region is

$$\bar{y} < 7.902 \quad \text{or} \quad \bar{y} > 8.098,$$

as seen in Fig. 3.2. The rejection region of Fig. 3.2 is given in terms of the test statistic \bar{Y} , the sample mean.

It is computationally more convenient to express the rejection region in terms of a test statistic that can be compared directly to a table, such as that

Figure 3.2
Rejection Region for
0.05 Significance



of the normal distribution. In this case the test statistic is

$$Z = \frac{\bar{Y} - \mu}{\sigma/\sqrt{n}}$$

$$= \frac{\bar{Y} - 8}{0.05},$$

which has the standard normal distribution and can be compared directly with the values read from the table. Then the rejection region for this statistic is

$$z < -1.96 \text{ or } z > 1.96,$$

which can be more compactly written as $|z| > 1.96$. In other words we reject the null hypothesis if the value we calculate for Z has an absolute value (value ignoring sign) larger than 1.96.

Step 3 of the hypothesis test is to collect the sample data and compute the test statistic. (While this strict order may not be explicitly followed in practice, the sample data should not be used until the first two steps have been completed!) In Example 3.3, suppose our sample of 16 peanut jars yielded a sample mean value $\bar{y} = 7.89$. Then

$$z = (7.89 - 8)/0.05 = -2.20, \text{ or } |z| = 2.20.$$

Step 4 compares the value of the test statistic to the rejection region to make the decision. In this case we have observed that the value 2.20 is larger than 1.96 so our decision is to reject H_0 . This is often referred to as a "statistically significant" result, which means that the difference between the hypothesized value of $\mu = 8$ and the observed value of $\bar{y} = 7.89$ is large enough to be statistically significant.

In Step 5 we then conclude that the mean weight of nuts being put into jars is not the desired 8 oz. and the machine should be adjusted.

The Five Steps for Example 3.3

The hypothesis for Example 3.3 is summarized as follows:

Step 1:

$$H_0: \mu = 8$$

$$H_1: \mu \neq 8$$

$$\alpha = 0.05.$$

Step 2: The test statistic is

$$Z = \frac{\bar{Y} - 8}{0.2/\sqrt{16}}$$

whose sampling distribution is the standard normal. We specify $\alpha = 0.05$; hence we will reject H_0 if $|z| > 1.96$.

Step 3: Sample results: $n = 16$, $\bar{y} = 7.89$, $\sigma = 0.2$ (assumed);

$$z = (7.89 - 8)/(0.2/\sqrt{16}) = -2.20, \quad \text{hence } |z| = 2.20.$$

Step 4: $|z| > 1.96$; hence we reject H_0 .

Step 5: We conclude $\mu \neq 8$ and recommend that the machine be adjusted.

Suppose that in our initial setup of the hypothesis test we had chosen α to be 0.01 instead of 0.05. What changes? This test is summarized as follows:

Step 1:

$$H_0: \mu = 8$$

$$H_1: \mu \neq 8$$

$$\alpha = 0.01.$$

Step 2: Reject H_0 if $|z| > 2.576$.

Step 3: Sample results: $n = 16$, $\sigma = 0.2$, $\bar{y} = 7.89$;

$$z = (7.89 - 8)/0.05 = -2.20.$$

Step 4: $|z| < 2.576$; hence we fail to reject $H_0: \mu = 8$.

Step 5: We do not recommend that the machine be readjusted.

We now have a problem. We have failed to reject the null hypothesis and do nothing. However, remember that we have not proved that the machine is working perfectly. In other words, *failing to reject the null hypothesis does not mean the null hypothesis was accepted*. Instead, we are simply saying that this particular test (or experiment) does not provide sufficient evidence to have the machine adjusted at this time. In fact, in a continuing quality control program, the test will be repeated in due time.



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Step 3: $p = P(|Z| > 2.20) = 0.0278$; hence the p value is 0.0278. Therefore, we can say that the probability of observing a test statistic at least this extreme if the null hypothesis is true is 0.0278.

One feature of this approach is that the significance level need not be specified by the statistical analyst. In situations where the statistical analyst is not the same person who makes decisions, the analyst provides the p value and the decision maker determines the significance level based on the costs of making the type I error. For these reasons, many research journals now require that the results of such tests be published in this manner.

It is, in fact, actually easier for a computer program to provide p values, which are often given to three or more decimal places. However, when tests are calculated manually we must use tables. And because many tables provide for only a limited set of probabilities, p values can only be approximately determined. For example, we may only be able to state that the p value for the peanut jar example is between 0.01 and 0.05.

Note that the five steps of a significance test require that the significance level α be specified before conducting the test, while the p value is determined after the data have been collected and analyzed. Thus the use of a p value and a significance test are similar, but not strictly identical. It is, however, possible to use the p value in a significance test by specifying α in Step 1 and then altering Step 3 to read: Compute the p value and compare with the desired α . If the p value is smaller than α , reject the null hypothesis; otherwise fail to reject.

ALTERNATE DEFINITION 3.10

A p value is the probability of observing a value of the test statistic that is at least as contradictory to the null hypothesis as that computed from the sample data.

Thus the p value measures the extent to which the test statistic disagrees with the null hypothesis.

EXAMPLE 3.4

An aptitude test has been used to test the ability of fourth graders to reason quantitatively. The test is constructed so that the scores are normally distributed with a mean of 50 and standard deviation of 10. It is suspected that, with increasing exposure to computer-assisted learning, the test has become obsolete. That is, it is suspected that the mean score is no longer 50, although σ remains the same. This suspicion may be tested based on a sample of students who have been exposed to a certain amount of computer-assisted learning.

Solution The test is summarized as follows:

1.

$$H_0: \mu = 50,$$

$$H_1: \mu \neq 50.$$

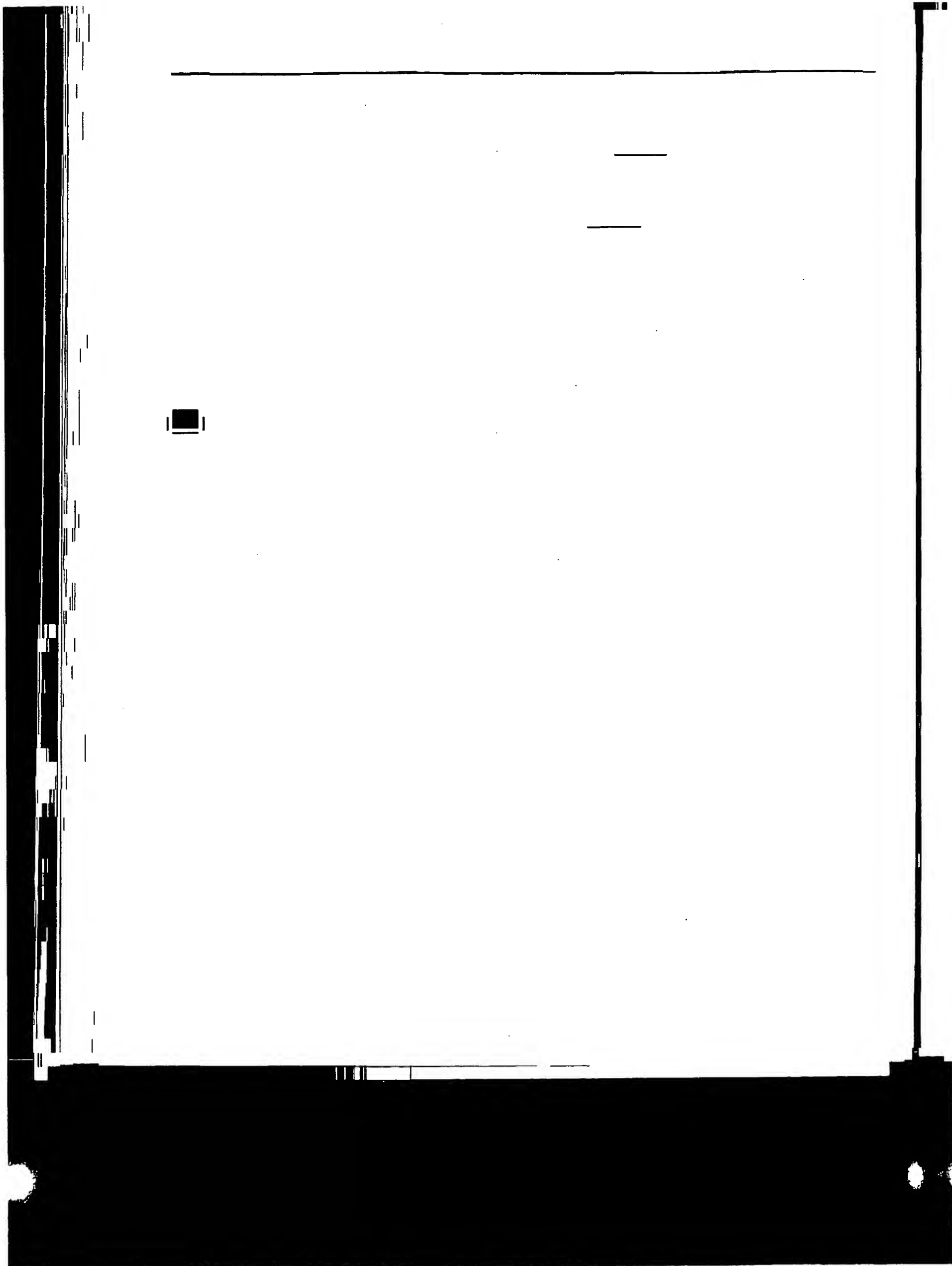


Figure 3.3

Probability of a
Type II Error When
the Mean is 7.95

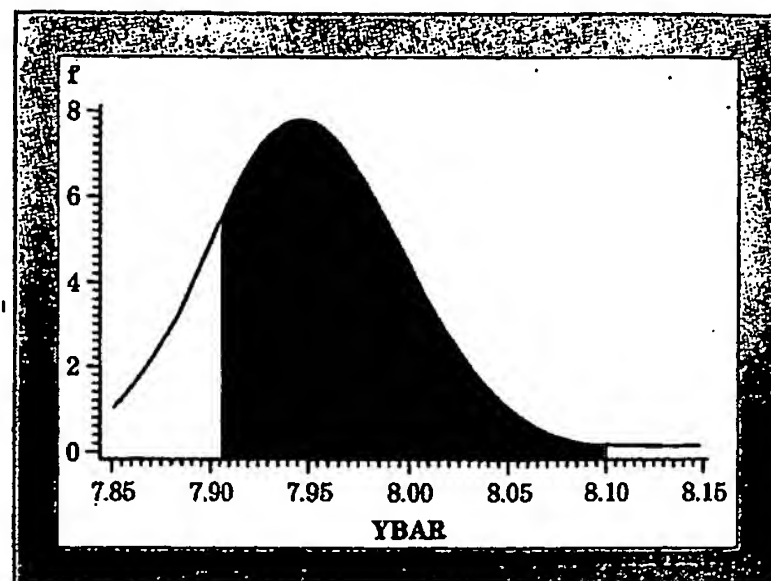


Figure 3.3 shows the sampling distribution for the mean if the population mean is 7.95 as well as the rejection region (nonshaded area) for testing the null hypothesis that $\mu = 8$. The type II error occurs when the sample mean is not in the rejection region. Therefore, as seen in the figure, the probability of a type II error when the true value of μ is 7.95 is

$$\begin{aligned}\beta &= P(7.902 \leq \bar{Y} \leq 8.098 \text{ when } \mu = 7.95) \\ &= P\{[(7.902 - 7.95)/0.05] \leq Z \leq [(8.098 - 7.95)/0.05]\} \\ &= P(-0.96 \leq Z \leq 2.96) = 0.8300,\end{aligned}$$

obtained by using the table of the normal distribution. This probability corresponds to the shaded area in Fig. 3.3.

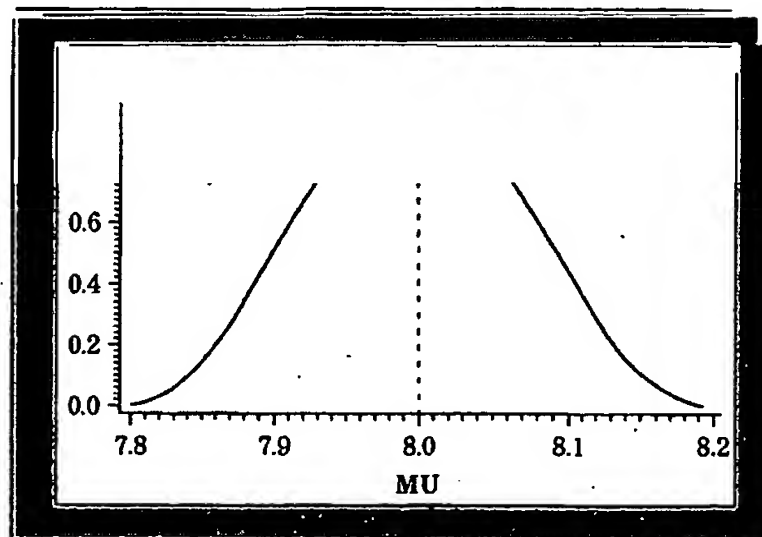
Similarly, the probability of a type II error when $\mu = 8.05$ is

$$\begin{aligned}\beta &= P(7.902 \leq \bar{Y} \leq 8.098 \text{ when } \mu = 8.05) \\ &= P\{[(7.902 - 8.05)/0.05] \leq Z \leq [(8.098 - 8.05)/0.05]\} \\ &= P(-2.96 \leq Z \leq 0.96) = 0.8300.\end{aligned}$$

These two values of β are the same because of the symmetry of the normal distribution and also because in both cases μ is 0.05 units from the null hypothesis value. The probability of a type II error when $\mu = 7.90$, which is the same as that for $\mu = 8.10$, is calculated as

$$\begin{aligned}\beta &= P(7.902 \leq \bar{Y} \leq 8.098 \text{ when } \mu = 7.90) \\ &= P(0.04 \leq Z \leq 3.96) = 0.4840.\end{aligned}$$

In a similar manner we can obtain β for $\mu = 7.80$ and $\mu = 8.20$, which has the value 0.0207.



While it is impossible to make a type II error when the true mean is equal to the value specified in the null hypothesis, β approaches $(1 - \alpha)$ as the true value of the parameter approaches that specified in the null hypothesis. The OC curve can now be constructed using these values. Figure 3.4 gives the OC curve for Example 3.3. Note that the curve is indeed symmetric and continuous. Its maximum value is $(1 - \alpha) = 0.95$ at $\mu = 8$, and it approaches zero as the true mean moves further from the H_0 value. From this OC curve we may read (at least approximately) the value of β for any value of μ we desire.

The OC curve shows the logic behind the hypothesis testing procedure as follows:

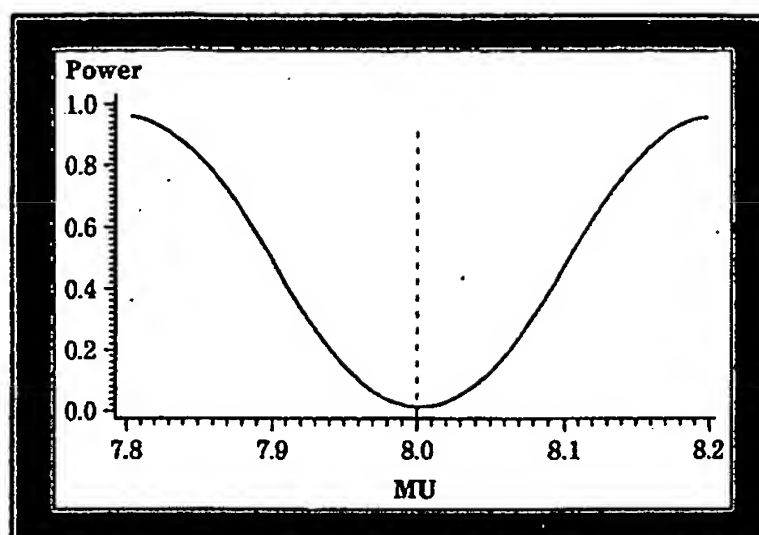
- We have controlled the probability of making the more serious type I error.
- The OC curve shows that the probability of making the type II error is larger when the difference between the true value of the mean is close to the null hypothesis value, but decreases as that difference becomes greater. In other words, the higher probabilities of failing to reject the null hypothesis occur when the null hypothesis is "almost" true, in which case the type II error may not have serious consequences.

For example, in the peanut jar problem, failing to reject simply means that we continue using the machine but also continue the sampling inspection plan. If the machine is only slightly off, continuing the operation is not likely to have very serious consequences, but since sampling inspection continues, we will have the larger probability of rejection if the machine strays very far from its target.

Power

As a practical matter we are usually more interested in the probability of not making a type II error, that is, the probability of correctly rejecting the null hypothesis when it is false.

Figure 3.5
Power Curve
for Example 3.3



DEFINITION 3.11

The power of a test is the probability of correctly rejecting the null hypothesis when it is false.

The power of a test is $(1 - \beta)$ and depends on the true value of the parameter μ . The graph of power versus all values of μ is called a power curve. The power curve for Example 3.3 is given in Fig. 3.5. Some features of a power curve are as follows:

- The power of the test increases and approaches unity as the true mean gets further from the null hypothesis value. This feature simply confirms that it is easier to deny a hypothesis as it gets further from the truth.
- As the true value of the population parameter approaches that of the null hypothesis, the power approaches α .
- Decreasing α while keeping the sample size fixed will produce a power curve that is everywhere lower. That is, decreasing α decreases the power.
- Increasing the sample size will produce a power curve that has a sharper "trough"; hence (except at the null hypothesis value) the power is higher everywhere. That is, increasing the sample size increases the power.

Uniformly Most Powerful Tests

Obviously high power is a desirable property of a test. If a choice of tests is available, the test with the largest power should be chosen. In certain cases, theory leads us to a test that has the largest possible power for any specified alternative hypothesis, sample size, and level of significance. Such a test is considered to be the best possible test for the hypothesis and is called a "uniformly most powerful" test. The test discussed in Example 3.3 is a uniformly most powerful test for the conditions specified in the example.

The computations involved in the construction of a power curve are not simple, and they become increasingly difficult for the applications in

subsequent chapters. Fortunately, the performance of such computations often is not necessary because virtually all of the procedures we will be using provide uniformly the most powerful tests, assuming that basic assumptions are met. We discuss these assumptions in subsequent chapters and provide some information on what the consequences may be of nonfulfillment of assumptions.

Power calculations for more complex applications can be made easier through the use of computer programs. While there is no single program that calculates power for all hypothesis tests, some programs either have the option of calculating power for specific situations or can be adapted to do so. One example using the SAS System can be found in Wright and O'Brien (1988).

One-Tailed Hypothesis Tests

In Examples 3.3 and 3.4 the alternative hypothesis simply stated that μ was not equal to the specified null hypothesis value. That is, the null hypothesis was to be rejected if the evidence showed that the population mean was either larger or smaller than that specified by the null hypothesis. For some applications we may want to reject the null hypothesis only if the value of the parameter is larger or smaller than that specified by the null hypothesis.

Solution to Example 3.1 In the example at the beginning of the chapter, we were interested in determining whether leasing office space in Atlanta costs more than that in Jacksonville. If we let μ be the mean cost per square foot of office space in Atlanta, and if we assume the standard deviation of costs is the same in both cities ($\sigma = 4.50$), we can answer the question by testing the hypothesis⁶

$$H_0: \mu = \$12.61,$$

$$H_1: \mu > \$12.61.$$

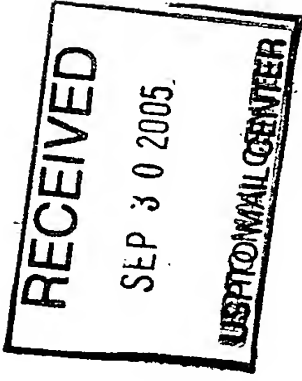
Note that the alternative hypothesis statement is now "greater than." Even though the possibility exists that the cost may be less in Atlanta than in Jacksonville, we really don't care. That is, the decision to move is to be based on the condition that the cost is higher in Atlanta. The businessman will stay in Atlanta if it costs no more to stay. The test statistic is calculated as before: $z = (13.55 - 12.61)/(4.50/6) = 1.25$. However, in this case rejection of H_0 is logical only if the value of \bar{y} is larger than that specified by H_0 , which corresponds to positive values for the test statistic z . Thus the entire rejection region is in the upper tail. A test that locates the rejection region only in one tail of the sampling distribution is known as a "one-tailed" (or one-sided) test. For this example, we will let $\alpha = 0.10$, and the rejection value is $z = 1.28$ (the

⁶To be consistent with the specification that the two hypotheses must be exhaustive, some authors will specify the null hypothesis as $\mu \leq 12.61$ for this situation. We will stay with the single-valued null hypothesis statement whether we have a one- or two-tailed alternative. We maintain the exclusive and exhaustive nature of the two hypothesis statements by stating that we do not concern ourselves with values of the parameter in the "other" tail.

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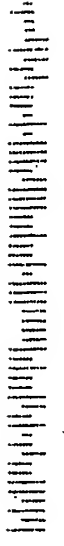
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